



12 July 1979

The Skylab syndrome

There has been something deeply symbolic about Skylab's ungainly descent back down to earth. After all, it is almost ten years to the day since the US successfully carried out what many consider to represent the pinnacle of its scientific and technological achievement: placing a man on the surface of the moon. The deed did, indeed, turn out to be a turning point, but perhaps not in the way that those responsible for the lunar programme intended. For it took place on the eve of a decade in which mankind has turned its technological concerns away from the stars and back to its own planet, and to the more mundane, but no less complex, concerns such as health, energy and natural resources that have become the new prime targets for research. Skylab's demise comes on the eve of a new decade; and its message, which seems likely to become a dominant political theme in the years ahead, is that high technology frequently carries a built-in risk factor which, if not adequately planned for, will return to haunt us with its own particular form of revenge.

The whole incident could not have occurred at a worse time for the National Aeronautics and Space Administration. The agency's public image is currently being seriously tarnished by a set of technical problems, and related cost over-runs, in the space shuttle development programme. These problems have forced the agency, for the first time in many years, to go back to Congress cap in hand to request a substantial increase in funding over what had previously been agreed. With Congress already doing what it can to reduce expenditure across the whole federal budget, NASA's case has not been helped by an incident which links the agency directly to other areas of public concern over the dangers of high technology, in particular the Three Mile Island accident and the DC-10 air crash. And although it seems likely that the extra money will be forthcoming — largely because of the military importance of space shuttle for launching satellites and carrying out surveillance tasks — other areas of NASA's activity may be made to suffer as a result.

At one level, both the problems with Skylab and the space shuttle can be attributed to the consequences of working with tight financial budgets. Skylab's designers saved \$10 to \$20 million by not adding engines that would have boosted the space station in its orbit when it began to slip back to earth, enabling it to be kept in orbit until it could be reached by space shuttle (the shuttle was initially expected to be launched this year, and Skylab to stay in orbit until 1983). And in the case of the shuttle itself, delays have been caused largely as a result of the development programme being planned according to a success-oriented strategy, with minimum back-up to allow for problems.

But it has not only been a question of money; there is also the question of control, and the associated attitude towards risk-taking. With space shuttle, the main contractor for the engine development has admitted to a certain laxity in overseeing the development of the programme, and has announced moves to remedy this situation by establishing close links between the

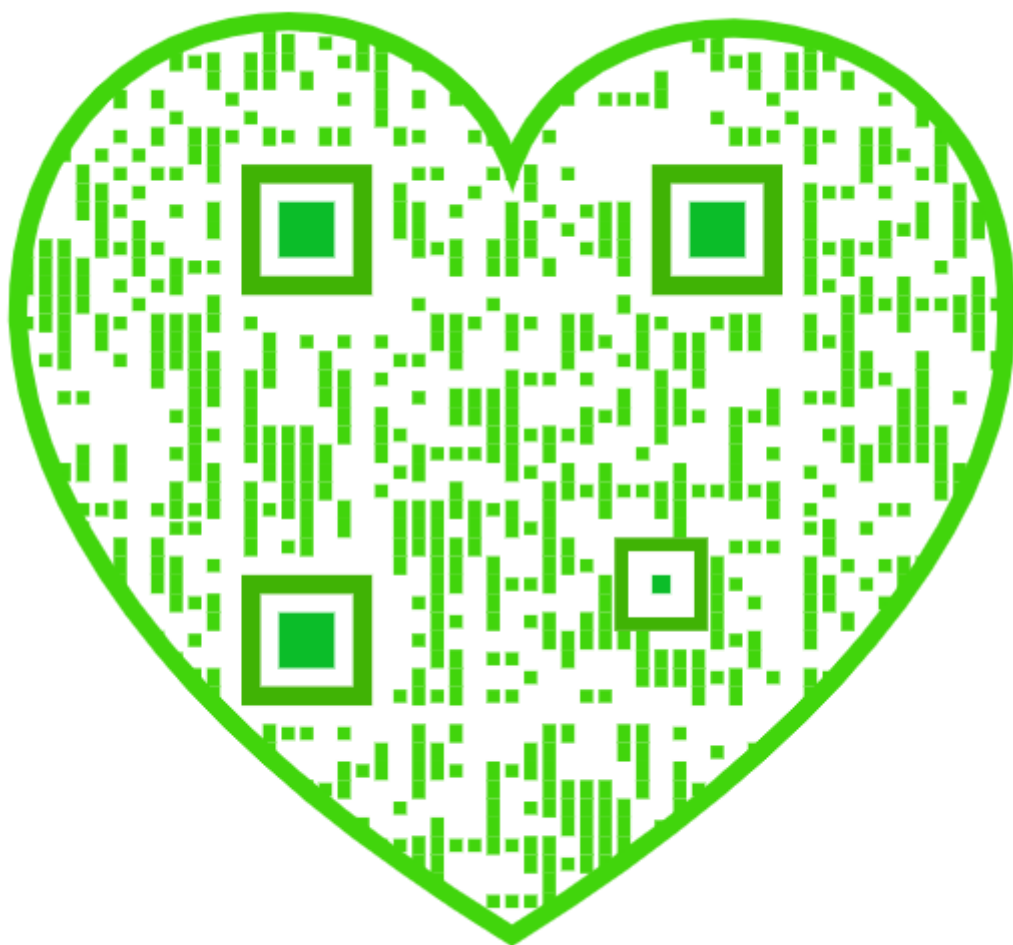
technical and the financial staff. In Skylab's case that lack of adequate control, manifest in a more direct sense, has given rise to problems far too late for anything to be done.

The lesson of both would, in different ways, appear to be obvious: that in developing high technology with a potentially significant social impact, mechanisms to ensure adequate control of the technology should be developed at the same pace as the technology itself. The problem is that although this prognosis has logic on its side, it runs directly into conflict with the ideology of a system which rewards technological ingenuity rather than social responsibility. The market-place, rather than the political process, has been treated as the proper forum for weighing risks against benefits; and those who have introduced new technologies have, as a result, felt no responsibility for the way that purchasers choose to make use of them. Indeed a surfeit of regulation — society's attempt to control the various non-market impacts of technology — has been increasingly criticised in recent years for its impact on the innovation process; and pressures are growing both in the US and, in line with the new government's current ideology, in the UK to return to the market place in its purest form as the prime regulatory agency.

Such programmes are, as their supporters point out, intentionally risky ones; only, they would argue, by encouraging people to take risks — and subsequently to reward those whose risks pay off — can vitality be reintroduced into the economic system. But such an approach is also predicated on the basis that the losers will suffer; and the particular concern generated by high technology is that the economic gains generated by the risk-taking of some will be outweighed, not so much by the losses of unsuccessful risk takers, but by the broader impact of failure upon those excluded from the decision-making on the risk in the first place.

What has concerned people most about Skylab is not so much the fact of its re-entry as the virtual total lack of control over its descent. Just as in the case of the Three Mile Island incident, it was the involuntary aspect of exposure to radiation emitted from the plant, rather than the actual level of exposure (compared, for example, to normal background radiation), that generated the fiercest complaints. Equally control cannot be reduced simply to a managerial or technical function, but must be seen to reflect a broad sense of responsibility to the whole community.

It would be a tragedy if the dominant message left by the Skylab incident, following as closely as it does the other high technology accidents that have occurred this year, merely frightened people away from such technology on principle. However if what comes through is that the control of technology is as important as the technology itself, then the lesson will have been worth-while. Perhaps this is the most significant theme to have emerged from recent debates about the concept of "appropriate" technologies, both in developed and developing countries; perhaps, too, it is one that we can learn to put into action in the decade ahead. □



UK research council hits back at 'horror lab' allegations

THE UK Agricultural Research Council last week held a press conference to present journalists with a defence of its use of animal experiments, after the *Sunday Mirror* had published 'shock' accounts (see right) of experiments at the ARC's principal laboratory, the Institute of Animal Physiology at Babraham near Cambridge. "Scientists transplant udders!" said the *Sunday Mirror*, and "Our verdict: vile".

But last week the director of the centre, Dr Barry Cross, countered that the story was "a statement about human reaction, not about animal cruelty". He questioned the credentials of the *Sunday Mirror*'s two witnesses of what went on at the institute. The first, Dr Cross said, "was a grade EW6 technician — the lowliest form of human life — no O-levels, no CSEs", and he was remembering 10 years after the event. The second "was a window cleaner or an asphalter who strayed from his proper job". To an expert, their reports "indicate pain and distress on the part of the unqualified observer".

Dr Cross's response was perhaps not calculated to enlist the sympathy of the journalists from the popular press (the *Star*, the *Express*, the *Sun*, the *Daily Mirror* and the *Sunday Mirror* were present); but the statement that followed was.

"I'm not here to defend cruel experiments on animals", Dr Cross said. "I was the first student president of the Union for Animal Welfare". Cruel experiments "would frustrate all our work — because we are studying healthy animals".

The experiments questioned by the *Sunday Mirror* involved udder transplants in goats and cows, to investigate secretions into the milk; permanent access to the animal's stomach, to investigate the digestion of the rumen; and an operation to bring a vein in the neck closer to the surface, to allow less painful injections to be made.

The ARC scientists have developed techniques, involving skilled surgery such as would "shame the National Health Service", which induces only mild post-operative pain while the wounds are healing, said Dr Cross. After that, the animals enjoy "a life of 5-10 years of cosseted living".

Stress levels in the animals are remarkably low. A researcher attempting to measure cortisol levels — a stress indicator — in Babraham cattle was shocked to discover amounts well below textbook levels. At first he thought that the Babraham cattle must be of a particularly phlegmatic strain, thus disqualifying many previous experiments, but then the cortisol shot up to the standard level when a noisy visitor entered the room. "Animals are

more stressed on a farm than in our laboratory", said Dr Cross. "Our animals are the friendliest I know. They don't expect hostile people."

Moreover, said Dr Cross, referring to the case of a goat udder transplant, which appears to have caused most public reaction, a new-born kid actually prefers the transplanted teat, "and he must be considered unbiased".

"We have never been refused a Home Office licence" said Dr Cross. (The Home Office controls animal experiments through the 1876 Cruelty to Animals Act.) "I've only been a director at Babraham for five years; previously I've worked in seven laboratories in the US, Scandinavia, and the UK, and I've visited many hundreds of others, and I have never seen a standard of care that surpasses or even equals Babraham's."

Dr Cross also defended the Babraham work on scientific, economic, and medical grounds. "One in seventeen women suffer from breast cancer", said Dr Cross, "but ruminants do not. Our experiments may give a clue to what predisposes women to this." Tranquillisers derived from experiments in the 1940s and 1950s on the effects of drugs on animal brains. The contraceptive pill based on hormones secreted by the ovary, a lot of work on which was done at Babraham. Furthermore, "there is a lot of evidence" that the brain is altered by sex hormones at a very early period, and there is a question of injurious effects from the mother's milk if the mother is on the pill. The basic work on the udder at Babraham may shed light on this, said Dr Cross.

More direct agricultural benefits have been: pregnancy tests, synchronisation of oestrus in herds of cows to save veterinary labour, the use of prostaglandin to induce pigs to give birth in daylight (which reduces mortality), the improvement of animal welfare in intensive production, a reduction in methane production in the digestion of cattle (so lowering energy



costs), and the protection of plant protein in the rumen (so increasing the conversion efficiency of plant to animal food). "If you lock up Babraham, you lock up these advances", said Dr Cross.

It was also "a complete myth" that Babraham concealed its work. The institute has produced 2,700 scientific publications, and nine biennial reports — with photographs. Large numbers of the public have been admitted — 12 parties a month of students, farmers, foreign correspondents "and all sorts of layman" pass through. In the past 18 months there have been four visits from journalists; four TV teams; and three broadcasts.

Nevertheless Dr Cross refused to allow *Sunday Mirror* journalists, whom he called "propagandists", or photographers into the laboratory; but there was an agreement to allow an observer acceptable to both sides. Subsequently (8 July) the *Sunday Mirror* commented: "He is really saying to the public: 'It's none of your business, and you are too ignorant to be told the facts anyway'".

Meanwhile, Jean Pink, organiser of the anti-vivisection group 'Animal Aid', has claimed that the *Sunday Mirror* is prepared to give further press coverage to the "diabolical tests", and will photograph demonstrators outside the Institute on Saturday.

Robert Walgate

Polish science writers go too far

Last month the Polish media celebrated the first anniversary of the flight of Cosmonaut Miraslaw Hermaszewski. Ironically, their special features coincided with a growing disillusion among Polish scientists not so much with the space programme itself but with what they see as its propaganda exploitation — notably in the case of the controversial 'Syrena' results.

'Syrena' was a Polish-designed experiment, carried out aboard the Salyut-6 space station, in order to obtain semi-conductor triple crystals of mercury, cadmium, and tellurium using a gradual

cooling method and of mercury, cadmium and selenium, using gas phase sublimation. In order to maintain controlled conditions of weightlessness, the production of the crystals, using the Soviet-built 'Splay' furnace, was carried out during the cosmonauts' rest period, lest undue motion disturb the space-craft.

Nevertheless, last autumn, Hermaszewski intimated to *Nature* that he had got up several times during the 'night' to ensure that the experiment was working properly, since the project, named after the symbol of Warsaw, had considerable national significance.

Not surprisingly, initial reports in the Polish media claimed the experiment as an unqualified success. Monocrystals of high homogeneity were it was said, obtained, such as would be totally impossible to produce under conditions on Earth. Then in February 1979, a meeting was organised in Krakow by the Japan Electro-Optics Laboratory for Polish users of its equipment.

A paper presented here by Warminski and Zahorowski of the Institute of Physics of the Polish Academy of sciences indicated that 'Syrena' had simply yielded a mixture of crystals of various compositions, in which the degree of homogeneity and the composition of the specimens depended on experimental parameters such as temperature gradient.

This would not in itself have disconcerted the scientists — a negative result is also of scientific value — had they not felt the results had been deliberately exaggerated by the media, which had claimed the alleged hyper-homogeneous triple crystals as a triumph for Polish science within the framework of "Interkosmos" (the Comecon space programme). "Science has become a state prostitute!" one young physics graduate put it.

Nor, apparently, was the disillusion confined to the lower echelons. According to a circumstantial report published by the "opposition" (i.e. underground) *Biuletyn Informacyjny KOR*, Professor Adam Gierek, the son of the party leader, remarked to a prestigious gathering of specialists that 'Syrena' has brought us back from space to Earth with a bump!"

The disappointment with 'Syrena' does not, however, extend to the Polish Academy of Sciences. Dr R. Galazka, of the Institute of Physics, who presented the results of the 'Syrena' experiments at the European Space Agency Conference in Grenoble in April, told *Nature* that "it was not our aim to produce crystals for some specific purpose, but to check the process of crystallization in space. We were able to use a diffusion controlled technique, impossible on Earth, to get crystals with some parts up to 50% homogeneous. Also we were able to study the problem of cavitation under pressure. We never aimed at getting a single crystal — indeed the technique of the experiment made this impossible. But we did get some 5mm single crystal blocks".

Professor Stanislaw Grzedzielski, of the Academy's Institute of Space Research, had just returned from a discussion with Dr Jan Kaczmarek, Academic Secretary of the Academy (a post which carries Ministerial rank) on the future of the space programme. He told *Nature* that plans for 1981-85 would include projects taking into account the results of Syrena. These results, he said, were not strictly speaking a surprise "because we simply did not know what to expect. Had we known, we need not have done the experiments."

Any exaggerated claims in the media, he said (and he himself always avoided popular science features), must have been due to patriotic zeal on the part of the reporters and/or their inability to understand the nature of the experiment. Even in a "controlled" press, he intimated, such divergencies can and do occur.

He himself, although not an expert in materials science, was not unhappy with the results. "Had they simply obtained perfect monocrystals every time", he said, "then there would be nothing more to find out, and the matter would no longer be one of space science but only of space technology. As it is, we can do a lot more experiments. Indeed it is expected that the Americans will try to replicate our work."

"Now that the work has started," he added, "it is far too valuable to stop. And it is very cheap, too. You see, it is not true that it involves 'cosmic sums of money' as some people would like one to believe. We don't pay for the 'ride' at all — the place on the rocket, the launch, ground control systems and so on. We only pay for the

apparatus produced in Poland, and the associated data processing etc."

The Soviet Union, he explained, were so far prepared to provide launch facilities free. "Fraternal solidarity is very important to them. If you are a big power and want your neighbours as friends you have to give them something they wouldn't have had otherwise".

This assurance, if passed on to the Polish public, would doubtless allay the rumours that 'Syrena' cost "several tens of millions of dollars" (from Poland's scanty hard-currency reserves) — if, of course the public were to believe it. Disillusion with the reporting of 'Syrena' however, bids fair to percolate from scientific circles to the wider public — carrying with it a disillusion with all forms of science reportage. It is perhaps not without significance that at this year's meeting of the Polish Academy of Sciences, a determined effort was made to bring forward a resolution calling for an end to press censorship — a motion which was thrown out by an amendment proposed from the chair. **Vera Rich**

Mystery beams affect UK satellite

BRITISH scientists are mystified by signals emanating from regions around British Columbia and the Caspian Sea that are turning off high voltage supplies in the UK research satellite, Ariel 6. After each satellite orbit over the areas, the high voltage of two experiments is switched off while a third supply is completely unaffected. The commands controlling the switching consist of a 148.25MHz carrier with a 5kHz subcarrier carrying the command signal so the interference has to be highly specific in order to affect two supplies but not the third. Dr Len Culhane, Chief Project Scientist in Mullard Space Science Laboratory's X-ray astronomy group has no explanation. "We think the signals are being generated spuriously but we can't figure out how."

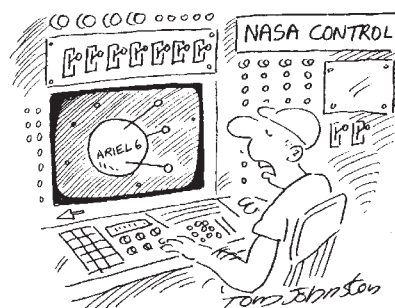
Five additional NASA ground control stations have been brought into play to turn the switches on after the satellite passes through the regions. Culhane is hopeful that eventually the satellite can be brought up to 80% of its operational capacity by further interventions.

Two other features of the interference puzzle the experimenters. First the interference occurs only when the Sun is shining on British Columbia or the Caspian. And second the experimenters have discovered that the satellite can be "immunised" against being switched off by beaming pure carrier frequency at it just before it enters the mysterious zones. The satellite orbits are such however that the satellite passes close enough to a neighboring ground station only 30% of the time — thus it cannot be immunised completely. Alan Wells of the Mullard group thinks that the likely cause is a jolt effect — a ground based radio signal combines with electrical noise in the space

craft to produce coding signals that activate the equipment switches. The possibility that the interference has been caused by vhf blasting signals coming from a dam construction site has been ruled out as has the possibility that interference is random.

In spite of the mystery the spacecraft is taking useful data. A Bristol University experiment on ultra heavy cosmic ray nuclei ($26 < Z < 92$) using a gas Cerenkov counter has been unaffected. The two X-ray experiments, one by Leicester University and the other a Mullard-Birmingham University collaboration has been fortunate enough to observe an X-ray burst from the X-ray source 1735-444 which was one of the hoped for observations of the mission. However a solar panel experiment designed by the Royal Aircraft Establishment at Farnborough has been unable to operate.

But short of some unexpected revelation it is likely that the reason for the interference will not be found, leaving the possibility that a space variant of the Bermuda triangle legend may be in the making. **Joe Schwartz**



"As it's a British satellite, it's probably just taking a tea-break!"

UNCSTD success still hangs in the balance

David Dickson reports from New York on final preparations for the UN Conference on Science and Technology for Development

With less than six weeks to go before official proceedings open in Vienna, a cloud of uncertainty still hangs over the chances of any major achievements coming out of the United Nations conference on Science and Technology for Development.

Delegates attending the fifth and final meeting of the conference's preparatory meeting in New York last week managed to reach agreement on a number of proposals that will be put to the conference as part of a 'plan of action' which the conference will be urged to adopt. However there are still major differences in three of the key areas to be discussed — the transfer of technology, and the institutional and financial procedures necessary to implement the decisions of the conference — and the stage has now been set for some hard bargaining over these in Vienna.

In general, the developing countries are seeking ways to improve their bargaining position over acquiring technology from the industrialised countries, to gain additional funds from these countries to build up their scientific and technological efforts, and to make the UN system more responsive to their needs in these areas by increasing their control over the relevant decision-making processes. In contrast, the industrialised countries, quoting domestic, economic, and political problems of their own, are keen to discuss specific areas of scientific and technological assistance, but have so far been digging their heels in over agreeing to any major procedural changes or financial commitments.

Most of the two-week session was spent by delegates in closed drafting meetings discussing, paragraph by paragraph, proposals for a new plan of action. The plan has been compiled by the Group of 77 (to which over 120 developing countries now belong) as an alternative to proposals put forward by the conference secretariat.

Agreement was reached on a number of proposals, mostly on relatively uncontroversial areas dealing with the internal arrangements that developing countries should make in order to increase their scientific and technological capabilities.

There were difficulties, however, whenever a paragraph arose that involved — or seemed to involve — the developed countries making any type of commitment to change existing arrangements. The developing countries have essentially used the proposed plan of action as the occasion to make a detailed statement of principle on what, they feel, should be done by developed countries and the United Nations bodies in the area of science and technology for development. In contrast, the developed countries — with a few notable exceptions, particularly the Nordic

countries — claim that such statements imply definite commitments of the type which many of the negotiators said they were unable to make.

The result is that when delegations arrive in Vienna, they will be faced with a series of documents liberally peppered with square brackets containing alternative formulations of key proposals. For example, where the Group of 77 suggests that the plan of action say "developing countries should be able to play a more effective role at the decision-making level in international organisations", the EEC suggests the same paragraph should read "all countries should . . .". Similarly, where the developing countries are demanding "full access" to western technology, the developed countries want this changed to "freest and fullest possible" access, a qualifier that dilutes the initial intent.

Three areas of disagreement remain. The first is the general area of technology transfer. Here the developing countries, in line with the stance taken in other UN forums, are demanding substantial concessions in their terms of access to the technology of the developed world. Industrialised countries, in contrast, are refusing to make any significant concessions, arguing partly that this is the responsibility of the private sector over which they have no control, and also that the issues are already being dealt with sufficiently elsewhere. The second area of disagreement is over the financial support needed to carry out the conference's recommendations. The Group of 77 argue that this can only be done with a considerable increase in the transfer of funds from the developed countries. They have suggested setting up a new financing system, largely financed by the industrial world, with a provisional target of \$2 billion by 1985 and \$4 billion by 1990.

The industrialised countries, however, have made clear their reluctance to commit themselves to any increase in such contributions. They are particularly critical of the suggestions that they be required to make "automatic" contributions to such a fund on the basis of, for example, their trade surplus in manufactured goods with the developing world.

Financial issues are also linked to the third area of disagreement, namely what changes should be carried out in the UN system to implement the conference proposals. The developed countries would like to see the strengthening of existing bodies, in particular the UN's economic and social council, to do this, although accepting that the status of science and technology should be raised within general

development strategies.

In contrast, the developing countries, distrusting what they consider to be the undue dominance of the industrialised countries in ECOSOC, want a new intergovernmental body to be set up. This might report directly to the General Assembly, with an executive general secretariat possibly established under the recently created UN post of Director-General for Development and International Co-operation.

A critical factor in this debate is likely to be the United Nations Development Programme. Although it has so far done relatively little explicitly concerned with science, UNDP has both wide experience of development issues and a high credibility among third world countries, and it may well be given operational responsibility, with an increase in funds, for decisions made by the intergovernmental committee in line with UNCSTD's final recommendations.

One phenomenon noted by a number of delegates at the meeting was an apparent softening of the US negotiating tactics. According to those who attended the closed drafting sessions, whereas the US had previously been pushing a relatively hard line in negotiations, disputing even relatively small points, they have subsequently slightly relaxed their stance, leaving the hard line more to the EEC countries ("and the British were the toughest of the lot" said one South American delegate).

Some have suggested that this shift in tactics may partly reflect a change of strategy by the transnational companies, eager to maintain their links with third world markets, but coming under increasing pressure from third world governments (particularly after the failures of UNCTAD V in Manila), and being forced to be more conciliatory.

Given the uncertainties that still remain, delegates leaving New York expressed views ranging from "concerned pessimism" to "cautious optimism" about chances of a significant agreement on these issues being made in Vienna. Much will depend on what happens in the negotiations between now and the end of August. The Group of 77 is meeting in Bucharest to discuss its negotiating strategy at the conference and others are meeting elsewhere.

On one point, however, the secretariat had its way. On the first day of the conference, the initial proceedings will include a performance of Beethoven's *Fidelio* overture. Some had complained that this would be time-wasting; others that, as western music, it might not be appreciated. The secretariat has defended its choice by pointing to the music's radical theme — and after all, you can't go to Vienna without listening to music. □

news in brief

UK government scientists step up strikes: British scientists are calling a further series of half-day strikes and demonstrations in support of their pay claim of 36%-47%. The government has offered increases ranging between 15% and 24%. The Institution of Professional Civil Servants is calling out its 20,000 scientists, 40,000 technicians and 10,000 related staff in a series of Tuesday and Thursday actions that will last until 7 August. Government establishments at Southampton, Leeds, Bath, Reading, Newcastle, Birmingham, Bedford, Cardiff and Glasgow will be involved successively. "This campaign will stop everything in these localities", said an IPCS official. The half-day actions add to the small number of volunteer indefinite strikers who are seriously affecting the country's communications and power supplies. In addition, IPCS has announced withdrawal of "good will" from operations at the Ministry of Defence, Departments of Environment and Industry and the Atomic Energy Authority. Support for further action is strong among union members.

King's College principal says UK universities should be cut: Sir Richard Way, principal of Kings College, London University has called for a reconsideration of the number of universities in the UK. Speaking at the opening of the 150th anniversary exhibition of King's College, Sir Richard gave his support to government economies. "Your government has been elected to try to establish some control over public expenditure and this is an aim which I very strongly support. I don't personally believe that the university system as a whole is necessarily short of money." Sir Richard told *Nature* that university expansion in the last 15 years did not mean that the expansion should be defended. "Just because you have 45 universities doesn't mean that you need 45 universities" he said. "I am an advocate of pruning." But such a measure need not compromise the UK's research capability. "It would be desperately wrong to compromise research", he said.

Benn calls for UN energy organisation: Tony Benn, former Energy Secretary in the Labour Government, has called for the creation of a United Nations Energy Agency to help avoid "confrontational energy policies". Although OPEC has previously resisted this proposal because of their fears of price control, Benn feels that the world is slowly moving in that direction. The important thing is to bring OPEC, the West, the Communist countries (who export oil to the West) and the non-oil producing countries into an arena that can prevent confrontation, he said.

Speaking at the annual conference of the National Union of Mineworkers, Benn also called for a public debate on nuclear power development in the UK. Previous growth of nuclear power has been predicated on overall growth, he said. But with a forecast of negative UK growth and with the events of Harrisburg in mind, big nuclear plants, particularly pressurised water reactors, need full public discussion. "We need absolute satisfaction that expansion is necessary, that expansion must be nuclear and that leaks and waste disposal problems are solved before going ahead."

UK stand on whaling: The UK government will support the proposal for a moratorium on commercial whaling. Mr Alick Buchanan-Smith, Fishing Minister at the Ministry of Agriculture, Fisheries and Food, announced this on Monday at the opening of the 31st annual meeting of the International Whaling Commission. "Resumption of whaling", he said, "should only be reconsidered if evidence of recovery of stocks and improvements in the methods of killing justifies it." He also announced government proposal for immediate discussions with the European Community calling for a Community-wide ban on sperm whale imports. This greatly disappointed environmentalists who had expected the government to announce a ban at the meeting. Czech Conroy, for Friends of the Earth, said although they welcomed the government's support for a

moratorium on commercial whaling, they found it "totally illogical and unsatisfactory" that sperm whale imports would continue to be allowed. "The proposed discussions between the government and our partners in the EEC to achieve a community-wide ban on sperm whale imports will permit thousands more whales to be killed." Friends of the Earth, he said, would continue to press the government "hard and fast" to follow up these discussions.

France opens new information centre: A new data centre, with the purpose of providing French scientists with bibliographic information produced by the world's major documentation services, was opened at Valbonne near Nice at the end of last month. The capacity of the computerised system will be 10^{10} bytes which will allow access on-line to about 10 million references.

The Centre was built in the record time of six months at the special request of Giscard d'Estaing, the French President. The authorities had been concerned about the rapid development of data banks in the US, UK and Germany, which according to Pierre Aigrain, Secretary of State for Research, could lead to French "cultural alienation". There was also concern that French research is not properly represented in foreign data banks. The official reason for the creation of the new centre, however, is economic. French scientists have been making about 60,000 inquiries to US data banks each year at a cost of 15 million FF.

—From the staff of *La Recherche*

Chinese synchrotron construction slows: China's 50 GeV/c alternate gradient proton synchrotron, originally planned for completion by 1982, will not be finished until 1985. The announcement appeared in the Beijing (Peking) People's Daily in an article by Zhao Dongwan, a vice-chair-person of the State Scientific and Technological Commission, who appears to be in charge of the administrative side of particle physics in China. He wrote that work is proceeding on schedule for the experimental centre housing the accelerator, but the target date of 1982 announced at last year's National Science Conference has been put back to 1985 because China's national economy is being adjusted. "This means a higher standard" Zhao emphasised. Zhao also stressed the need to continue the implementation of the principle of self-reliance. The basic approach he wrote would be to send people abroad to study advanced accelerator technology while designing and building the equipment at home. China should not import equipment that she herself could produce.

In the meantime the Sino-American Joint Committee on High Energy Physics held its first meeting in Beijing on 11-12 June. This joint committee is the first of a kind which came into being after the signing of the Scientific and Technological Agreements between China and the US earlier this year. The two-day meeting was jointly chaired by Professor Zhang Wenyu, director of the High Energy Institute under Academia Sinica and by Dr J.E. Leiss, associate director of the Office of Energy Research in Washington.

—From T.B. Tang

Education in Chile in decline: The long term educational policy of the Chilean junta aims to eliminate entirely universal education. In a statement made in March, President Pinochet stated that the government intends to reduce educational opportunities to ensure that no more than a handful of children, whose parents can afford it, go to secondary school. In 1978, less than 70% of all children received an education compared to 92% under the Allende government. The education budget has been reduced from 17% of the total to 13.5%. In addition, the administration of many schools has been given over to business consortia who hire the teachers and plan the curriculum. The consortia receive tax benefits for providing "resources for educational purposes". Operation of all schools from primary to university level is under the final authority of the Commando de Institutos Militares.

France puts science under the microscope

Pierre Aigrain talks about his first year as France's science minister, which has culminated in a blockbusting survey of the whole of French science. **Judy Redfearn** reports

AT THE END of last month, a report entitled 'L'état des sciences et des techniques Françaises', was circulated to French scientists, administrators and politicians for their information and comments. A slim document, it is the summary of a study conducted by the Délégation Générale à la Recherche Scientifique et Technique and the Comité Consultatif de la Recherche Scientifique et Technique (*comité des sages*) under the auspices of the science minister, Pierre Aigrain. Two thicker tomes, one for physics and one for life sciences are to be published later. "As far as I know, it is the first time that any country or any government has tried to do a study of this kind", according to Pierre Aigrain.

Previous studies, he says, have compared the level of funding of a particular area of research in France with that of other countries. But "that's not what we have tried to do here". The aim has been to identify the strengths and weaknesses of each branch of research in France: to compare it internationally and assess its relevance to national economic and social objectives. "This is a critical study which is necessarily subjective. I expect that it is going to make us very unpopular". But it was felt necessary as a basis for formulating future science policies. The exercise is to be repeated annually, to begin with, and then at longer intervals as the methodology improves.

Good research, explained M. Aigrain, poses few problems. Even if it is not particularly relevant to national needs, provided that it is not too expensive, it should be supported and encouraged. Problems arise, however, "with sciences in which we are weak. There are those in which we are weak compared to the rest of the world and those in which we are weak but the rest of the world is no better". An example of the latter is human nutrition. France may be in the front row, but "that row is moving much too slowly compared to needs".

The greatest difficulties arise, however, when France lags well behind international competitors in a particular field. Sometimes the best decision will be to forget about those areas. But in some cases, a weakness can have "unfortunate, sometimes catastrophic consequences", not only on that field but also on neighbouring ones. An example is clinical pharmacology which is important for good pharmaceutical research.

Pierre Aigrain, together with the DGRST and the *comité des sages*, is now

faced with incorporating the main findings of the study into the eighth five year plan for 1980-85 and a ten-year research and development strategy, both currently under preparation. France has been running five year plans for science and technology since the end of the war: the idea is to plan fairly precisely in advance what research projects should be done during the next five years. The ten year strategy, however, is more of an attempt to formulate basic guidelines for government R&D "in a ten year perspective".

One problem the ten year strategy will address is that of providing enough jobs. After the expansion of the universities in the 1960s, France, like many industrialised countries, now faces the problem of continuing to provide work for young scientists. With only a modest growth in the real value of the science budget, it has had the choice of either freezing the number of posts available and providing each scientist with a good working budget, or increasing the number of posts and consequently reducing the money available for equipment and research. As the lesser of two evils, it has chosen the latter.

In 1975, the government decided to increase the number of research scientists employed in its laboratories by 3% per annum and to keep the level of the science budget in line with the average rate of government investment. "The rules for the next ten years will be of a similar kind, although more elaborate", says M. Aigrain.

"The training of scientists is a long term problem", he explained. "Not only does it take a long time to train them, it takes even longer to establish good training systems. We feel that although creating new jobs only makes our problem worse, from the point of view of the amount of money each scientist has to work with, it is a less serious problem and more easily reversible than the one we would have if we let the training systems die out".

A good budget could greatly improve the remaining problem. The 1980 budget, for example, is expected to be appreciably more than the one for this year. If it is, then the shortfall in funds per research worker could be remedied in "one or two years". A new training system, however, would take 15 years to establish "if we let the present one die out".

The 3% growth rate has by no means solved the jobs problem. The French universities system is suffering particularly badly from stagnation as a result of the earlier expansion — a situation made worse by the fact that very few scientists, even



Minister of Science, Aigrain: "it's a rather special sort of job."

compared to most other countries, take jobs outside academia. "Our mobility rate is very low. If a greater proportion of scientists employed in government laboratories moved to other sectors, including research in private industry, we could have our cake and eat it: we could increase the means at the disposal of each scientist and at the same time maintain our training system".

Mobility, however, is a problem of psychology — not only of scientists but also of prospective employers. "You can't change behaviour by law — at least not in a democratic society. Actions can only be indirect". The indirect actions France is currently taking involve giving advantages to scientists who move into industry and their employers.

It is also trying to revamp an old system whereby scientists can be put on "detached duty" in industry for a few years. "Even this has not been as successful as we would have expected because while the scientists are on detached duty they have not been getting promotion from their government employers. This is something which we are going to try to change."

Moving research scientists into industry, however, not only benefits the universities and government research laboratories; it can also bring valuable expertise and new ideas to industry which badly needs more contacts of this type. "On the whole French science and technology is holding its own", according to M. Aigrain, "but the contacts between it and the people who can apply it are very poor. In my opinion, it is a matter of personal contacts, which are insufficiently developed."

The solution does not lie with creating more government labs for applied research, however. "I've been in industry in charge of R&D in a major company and I know that doing industrial research efficiently implies an industrial environment"

Knowing how best to act to stimulate

greater mobility of scientists or greater cross-fertilisation of ideas with industry, involves judgement of human behaviour and a bit of applied psychology. Psychology, however, is not one of the hard sciences, says Aigrain, who was initially trained as a solid state physicist. But since he became science minister one year ago it has assumed a greater role in his working life.

Aigrain came to the post after a varied career in both science and administration. As a young physicist he was appointed by General de Gaulle in 1958 to the newly created *comité de sages* (CCRSST) — de Gaulle had wanted some young blood on the committee — and later through earlier connections as a naval officer, he was appointed head of an advisory committee concerned with defence research.

After a spell with responsibility for higher education and a five year term as head of the DGRST (1968-73), he moved into private industry to become general technical manager of Thomson. "I had planned that the rest of my active life would be spent as general technical manager of an industrial company. I have never been involved in active politics, so it was a very great surprise to me when the Prime Minister, Raymond Barre, asked me to enter the government in April 1978."

Pierre Aigrain is one of a handful of

French ministers who have never run for election. Another is André Giraud, the industry minister, under whose department science and technology officially come. The job of the science minister, however, is unique in the French government.

"I operate by delegation from the Prime Minister on inter-ministerial matters. My work mostly involves coordination, strategy, planning and budgeting of civilian R&D in all ministerial departments. This is a rather different job from that of a minister who has to run a department. What I have to do is convince my colleagues or the Prime Minister of what should be done in matters of science and technology. It's a rather special sort of job." The DGRST is directly under his responsibility. "DGRST is really my operating cabinet and my means of action."

Determining the science budget, and how it should be spent, is Pierre Aigrain's major task. France currently spends about 1.8% of its gross national product on R&D. Ten years ago, the figure used to be 2.1%; 20 years ago it was well below 1%. In spite of the decrease in this percentage over the past ten years, the volume of research has increased very slightly because of the modest growth in GNP.

On the whole, M. Aigrain believes that the French government is sympathetic to

the needs of science and technology. His main problem with parliament, however, is to explain "why, with the budget we have, we should put money on this and that and not necessarily on some of the ideas which are in the news — which may not be the best, or which anyway, cannot be efficient unless we also maintain a sufficient amount of basic research."

He agrees that parliament could be much better informed. A group of MPs, known as Intergroup, used to meet regularly to discuss science policy issues, but with the pressure of seemingly more urgent commitments, this has lapsed and there is now no equivalent of the UK select committee structure whereby MPs can gather the opinion of scientists at the grass roots. Something along these lines would be "very useful", however. Scientists "have a duty to try to explain to politicians what the problems of science are, not in party political, but in science policy terms: down to earth terms".

For one reason or another, France has missed out on many of the debates around science policy issues which have aroused public interest in recent years in other industrialised countries. In the case of nuclear energy, Pierre Aigrain thinks that the French people recognise that their country, having very few energy resources of its own, is in a particularly difficult position. "There may be many Frenchmen who don't like nuclear energy", he says, "but there are few who doubt that it is necessary".

In the case of genetic engineering, the French public recognised early on, the confusion surrounding the debate in other countries, where concern had centred on the possibility that "some experimentation could be dangerous for the people doing it or the immediate surroundings" and "the vague fear" that genetic engineering could lead to "ethically unacceptable" experiments. "In France I think it was clear from the start that these were two different problems. The problems of the dangers could obviously be handled provided that scientists took the right precautions. People are, I think rightly satisfied that we can handle this problem because we have been handling a much more difficult one for tens of years," — namely the production of large quantities of vaccine from live virus.

About health and safety generally, and concern over carcinogens and toxic substances, M Aigrain says that there are worries in France, but that "up until now, Frenchmen have been a little more rational and a little less emotional. We in France have a big drawback. We tend to have too much of an analytical mind — we are too rationalistic, too Cartesian. In research this is not necessarily an advantage because, in order to be creative, researchers have to be a lot more pragmatic. But it does have the advantage that even the average Frenchman can realise relatively easily that a debate is created on a non-logical basis".

What the report says about French science

TEN thousand people, of whom 4,500 are qualified scientists, are employed in fundamental physics research in France. The budget for paying their salaries and buying equipment for their work is about 1.7m FF per year. A further 3,500 researchers are employed in physics for engineering.

The DGRST study found French fundamental physics rather healthy, especially in the fields of high energy physics (where France has a reputation for building bubble chambers), nuclear physics, thermonuclear fusion and solid state physics. (The latter is an example of a fundamental branch of research which has good links with industry.) Gravitation, cosmic rays and molecular physics are weak, however.

In applied physics, says the report, the results of research in optics and fluid mechanics are finding their way into industry, but those in solid mechanics and materials sciences are not. The quality of research, for example, in materials sciences is good, but it is unevenly distributed amongst sub-fields and is too academic to be well tuned to the needs of industry. Chemical research done in universities and government laboratories, however, has close links with the chemical industry, but there is need for even greater collaboration. In quantum chemistry, radiochemistry, nuclear chemistry and catalysis (with the exception of catalysis by enzymes)

collaboration is good; but it is poor in electrochemistry and chemical engineering. Much of the research is very dispersed and there is a need for some regrouping.

About 4,500 full-time and 6,000 part-time scientists are employed in life sciences research. Areas of which France is particularly proud include cellular and molecular biology, endocrinology, applied immunology and and reproduction and development. Over the past seven years, the programme in the latter has led to a reduction in perinatal death from 23.4 to 16 per thousand. Bacteriology and human nutrition, however, are very weak and neurobiology is currently being 'rejuvenated'.

In energy research, a field with important social and economic implications, the report says that France is doing well in nuclear energy and solar power. It is going ahead in 1982 with plans to start building light water reactors under licence from Westinghouse and with the construction of its demonstration commercial fast breeder reactor, Superphenix. In renewable energy research, it is putting most of its efforts on solar energy and in the long term, thermonuclear fusion. Research on fossil fuels is strong when it comes to prospecting for off-shore for oil (France is well renowned for off-shore technology because of its efforts in this area) but weak when it comes to coal research.

Will China be tainted by western science?

CHINESE science is treading a careful tightrope between its long-term aspiration of a specifically Chinese-orientated scientific outlook and the short-term expediency of importing western ideas wholesale. Recent statements have indicated that although the ideal is still to the forefront of Chinese scientists' minds, it is perhaps being undermined by the very scientific exchanges essential to the modernisation of Chinese science.

The influx of western ideas into the physical sciences pertains to science itself as well as to its social dimension, which includes the administration, funding and general management of science and technology. In the past few months, some ministers and many scientific groups from China have toured scientific institutions in the west, and a large number of western scientific teams are returning the visits. The first impressions apparent in the media were that the Chinese showed great interest in western practice. An example is that after the visit of the State Scientific and Technological Commission delegation to Japan last December, articles appeared discussing the merits of such institutions as the patent system which were introduced into Japan when it became westernised. In fact, however, the Chinese attitude is a cautious one. Commentaries in the party paper on 19 April and 8 May, for instance, stressed that while China is learning advanced science and technology from abroad, it will on no account blindly import social practices; future science policy will be firmly China-based.

On the other hand, in looking towards the future, the Chinese realise that their present system of science management is

not perfect. They freely admit, among other things, that their national research efforts are plagued by wasteful duplications as well as damaging omissions — perhaps surprising in view of their central planning. It seems that the misguided motivation of some scientists, together with insufficiently democratic coordination between parallel funding offices, are the major factors. Some institutes have recently adopted the practice that individual contribution is judged mainly by the authorship of papers, but such professionalism can sometimes lead to researchers doing only publishable and prestige-promising work. The Chinese believe that the problem of rationalising the organisation of science and the decision-making processes will be solved by the inventiveness of the masses, rather than by imitating another country, no matter how advanced its science. Thus, many of the systems created and evolved in China during the last twelve years are likely to be kept, forming as they do a useful basis for improvement. During this period the achievements of Chinese science have been satisfactory, when compared to the resources available; last year, according to preliminary statistics, more than 12,000 research projects were successfully completed.

This attitude, that a specifically Chinese science can build on a firm past basis, is reiterated in a report on 10 April in the *Guangming Daily* of a meeting of Beijing University professors. The speakers, among them several leading scientists, asserted that as developments from foreign countries are incorporated into the Chinese social system, Chinese science must retain its original character. To us,

its outstanding features include an emphasis on combining both traditional and modern knowledge, on linking laboratory investigations with production in the field or factory, on collective rather than individual efforts and rewards, and on the participation of the masses in both the direction and the conduct of research. It is relevant in this context to note that China continues to devote considerable resources to the popularisation of science: it is setting up four additional specialised studios to cater for the rising demand for science and educational films, a demand at present partly satisfied by dubbing films purchased abroad. In the meeting, some professors also argued that political education should be emphasised in the training of young scientists.

For China, at present, the provision of scientific and technological manpower holds the key to the 'fourth modernisation' (*Nature* 19 April, p. 682). A concrete expression of this situation is that instruments such as electron microscopes — if they were foreign imports — are often under-used due to inadequate experimental skill or lack of maintenance.

According to a 1977 report from the Organisation for Economic Co-operation and Development in Paris, there were then some 335,000 scientists and 725,000 technicians working in China (excluding personnel engaged in medical research). At the National Science Conference convened in March 1978, the goal was set that by 1985 an army of 800,000 scientific workers will be formed, double the present number. Chinese universities took in a total of nearly 400,000 new students last year, and to help its educational targets China announced last August that it also plans to send some 10,000 students to the US, the UK, France, Germany and Japan. This year about 500 are going to the States, 200 to England, and 100 undergraduates have already arrived in France.

Although western visitors may import foreign ideas into China, the real danger to the concept of a long-term Chinese-orientated science lies in the return of these many young people after exposure to western scientific culture (although the flow in the opposite direction may also be interesting). This trend will be exacerbated by closer links between students in China and their counterparts abroad.

At present there is a dynamic balance between the ideal of an indigenous Chinese scientific culture and the influence of established western scientific ideas; only in the next five years, as these influences are absorbed, will the future pattern of the world's largest new scientific force become apparent.

T.B. Tang



The world's first giant panda born by artificial insemination made its debut at Peking Zoo recently. The panda, named Yuan Jing — meaning "First Crystal" — was born last September weighing only 120 grams. Yuan Jing has aroused interest around the world because of renewed hopes for the famous but unco-operative panda pairs at London Zoo and

Washington Zoo. All previous attempts to mate the pairs have failed, including flying in other pandas from the Soviet Union and China. China is the only country so far to have bred pandas successfully in captivity. Baby Yuan Jing started to walk at three months, developed the familiar black and white hair at five and was weaned at seven months.

Mexico: slow fight against impossible odds

By John Hutton

MEXICO City is one of the largest and fastest growing cities in the world. The colonial heart, built upon the remains of the Aztec capital, is beginning to atrophy as the periphery expands. The volcanic peaks which crown the valley are often hidden in the smog produced by industry and by the ageing vehicles which edge bumper-to-bumper down the arterial thoroughfares designed for a city of a smaller size.

The provision of services cannot cope with the influx of population. There is a chronic housing shortage and, in many parts, scanty supplies of water and electricity are further burdened by illegal tapping. Yet, every day, thousands of people pour into the city. They come to establish themselves in the ranks of power, to enter business, to seek higher education, to find jobs in the factories or to work as household servants.

Mexico City's population of about 13 million is expected to reach 30 million by the turn of the century, and its current rate of growth, the nation's population should exceed that of the USA in fifty years. While 30% of Mexicans enjoy incomes comparable to those in the developed world, more than 50% know the realities of underdevelopment.

Malnutrition is widespread, as is chronic respiratory and gastro-intestinal disease. Life expectancy is about 65 years but infant mortality is estimated at 66 per thousand live births, more than twice that of Cuba and four to five times that of the USA. More than half the population receive no more than a few years schooling and illiteracy stands at 25%. In recent years, the domestic production of foodstuffs has not sufficed and staples, including corn, have had to be imported.

In confronting these problems Mexico can call upon a stable political system, a diversified economy, the vision of its administrators and, in recent years, its vast reserves of petroleum.

In accordance with the spirit of her constitution, Mexico sees the key to development as lying in investment in education and in the construction of an economy with an indigenous base rather than one which relies on imported technologies. In keeping with these plans, investment in research and scientific education has burgeoned in the past ten years. And there now exist pockets of activity which, though not yet mature, are well on the way to manifesting a style which



Universidad Nacional Autónoma de México: increasing demand for education

is both in line with the country's aspirations to knowledge and relevant to its material needs.

The body largely responsible for the implementation of this policy has been the 'Consejo Nacional de Ciencia y Tecnología' (CONACYT) which was established in 1971 during the presidency of Luis Echeverría. In 1960 Mexico could boast only 385 qualified investigators which, given its rate of demographic growth and the scientific boom in the West, was little advance upon the 30 or so registered in 1929. By a system of grants and scholarships CONACYT brought that number up to 13,300 by 1978. And in the 1978-1982 period a further 17,000 scholarships will be awarded, most of which will be for postgraduate research within the country.

The pattern of the scholarship distribution reflects the priorities of the planners: 17% to studies on the use of energy resources, 13% to agronomy and forestry, 13% to basic research, 11% to nutrition and health, 9% to social development, 7% to fishing, 7% to construction and transport, and 2% to public administration.

This is a far cry from the climate of the 1950s and early 1960s when the glamour of imported technology held sway. In an era often described as 'anti-science' or 'anti-development', overseas-based corporations were given preference in the construction of manufacturing industries; in the field of agriculture the US-inspired 'green revolution' was promulgated; and even in the petroleum industry, nationalised since 1938, much of the geological data concerning the country's resources lay in foreign hands.

Movement away from this ethos gathered impetus in 1961 when the 'Instituto Mexicano del Petróleo' was set up. Apart from direct benefits to the industry, this initiative helped encourage the return of scientists from overseas. By the same token, it provided ammunition for those who wished to draw attention to the dearth of scientists within the country and the need for an adequate training

scheme. Meanwhile, in the universities there was a mounting realisation that the nature of the social system had to be changed, a movement which culminated bloodily in the student riots of 1968.

Cries for reform were heeded by the incoming president. In 1970 Echeverría made the unprecedented move of increasing spending on education from 1.8% to 3.1% of the Gross National Product (GNP). At the primary school level the text books were re-written with an emphasis on authentic growth. Science education was begun at the age of six and broadened to include such sensitive issues as human sexuality, a move which required presidential sanction and which met with fierce opposition from conservative sectors of the press and television. The CONACYT scholarship plan was instigated at this time, and spending on research radically increased, from 0.1% of the GNP in 1970 to 0.6% by 1978; it should reach 1.0% by 1982.

During the 1970-1978 period, a policy of support for basic research has evolved. The rationale has been that work of an applied nature would flow more soundly from a solid foundation of scientific principles, and that experience in basic research is essential to the attainment of a 'critical mass' of research facilities and expertise.

The pursuit of such a policy seems to have paid off, as indicated by the emergence of a new type of scientist: one whose work habits are geared to productivity, one who is open to new ideas rather than dedicated to perpetuating dogma, one who demands the critical participation of his students rather than their unswerving loyalty.

It will take time, however, before the new ventures in science make their full impact upon the economic and social development of the country. The successes attained so far have sometimes been marred by the difficulties of realising the economic potential of a discovery. As Dr Arturo Gomez-Pompa, director of the 'Instituto Nacional de Investigación sobre Recursos Bioticos', put it: 'the economic potential of a chemical with interesting

The author visited Mexico on a Nature travelling fellowship.

pharmaceutical properties, extracted from a native plant species, may be lost when the material falls into the hands of a foreign concern which modifies it, patents it and then markets the new compound".

Several ambitious attempts have been made to grapple with this problem, within the framework of a move towards decentralisation. In Tonanzincla a church in the style of Indian baroque, open fields and eucalyptus trees provide an idyllic setting for the 'Instituto Nacional del Astrophisica, Optica y Electronica'. Known in the village as the 'observatory', this once prestigious centre of astrophysical research is now a base for investigation into optics and electronics. Basic research is undertaken by the fifteen or so staff and by the fifty-odd students who are studying for higher degrees.

Alongside the laboratories a pilot factory has been constructed for the manufacture of microscope lenses. Grants are awarded to enable young people from the village to work on the production line.

At the University of Puebla technical expertise in the construction of micro-circuitry is also being developed for research and teaching purposes, in addition to its contribution to the nation's needs. As at Tonanzincla much of the equipment is manufactured at source.

In the State of Durango, the government has set aside a large tract of land representative of the eco-system of the region. This 'biosphere' is designed not only to preserve the genetic diversity of plant and animal species in the region but to provide a laboratory in which experimentation into more rational forms of land use can be made. This project is being conducted in collaboration with the local farmers of the area.

None of these achievements have been gained easily. The constitutional policy of a six year non-renewable presidency brings with it administrative re-shuffling and consequent re-formulation of plans. The scientist, like anyone else, has little guarantee of continuity in his work. Time must be invested in convincing each new administration of the validity of his ideas, either through the cultivation of friends in power or through the publication of his work in a form amenable to the layman. Good though this may be for the purposes of public debate it consumes much of the energy of the successful man.

The scientist must also face the problems of the nation as a whole. He must be able to cope not only with present-day realities but with the contingencies which emerge as the population increases in numbers, literacy, and as more than ever move towards the city. Can the 'Universidad Nacional Autonoma de Mexico', for example, with 150,000 students already, accommodate the increasing demand for higher education without sacrificing teaching standards? Many see it as the race against time.

The scientist, it is hoped, will be

All at sea

THE muscular American was ahead of me at the cashier's desk in the hotel at Delhi on 3 June. He was arguing forcefully about charges on his bill for more than the actual number of local telephone calls that he said he had made. This auditing impressed me as being unusually precise, but perhaps, I thought, he is a professional accountant. The hotel won a computerized victory, because the local calls were metered to each room.

A few minutes later, I stood beside him in the front entrance to leave for the airport, and I noticed that his luggage was tagged "Agency for International Development". Spontaneously, I spoke to him; I said that I was interested in the public health work of AID, especially in malaria. He said that his was a different field, that of disaster relief. He invited me to ride to the airport, in his officially-provided car, where we would take the same flight, I to Bangalore, but he to an earlier stop, Hyderabad. He had to organize relief for victims of the 11 May cyclone that had devastated a large area in Andhra Pradesh.

I asked him what he had done lately, and he said he had recently been to Yugoslavia to help the survivors of an earthquake. He then launched a discussion of rehabilitation, how, for example, it was better that an Indian farmer receive a live replacement for a dead bullock rather than a gift of food. A variant, I thought, of the old adage about helping a starving man to catch fish.

George Beauchamp was my companion's name, and obviously he was used to taking charge. He deftly piloted me through the churning crowds that pack Indian airports and through the check-points to the departure area. We boarded the air-bus and went to our separate seat assignments after bidding each other goodbye.

As I sat in the plane, I suddenly realized what George Beauchamp must really be like. The surface that he had shown to me was that of a brisk, efficient and business-like public employee, arguing about his bill, and carefully attending to other details of the day. Impulsively, I got out of my seat, and went to find him. "George", I said, "I wish you all future success in your great work for humanity".

He thanked me, and then poured out his indignation at the current treatment

instrumental in finding alternatives to mitigate the flood of people reaching the city, people who, each in their vague search for opportunities, constitute a major flight from reality when considered within the nation as a whole. The heroes of Mexican folklore are not Cortes and his men but



Thomas H. Jukes

of Vietnamese refugees, adrift on a hopeless sea, rejected at all attempts to land. He said that a few years ago he had assured people in Vietnam, in answer to their doubts, that the President of the United States would not let them down. "And now," I replied, "the Americans have forgotten the poem by Emma Lazarus". In answer, he passed me the book he was reading: It was the historical account of the ship that carried doomed and despairing refugees from Hitler from port to port, and finally back to extermination in the gas-chambers of Germany. George pointed to a verse on the front page; there were the wellknown lines I had just quoted to him:

*"Give me your tired, your poor,
Your huddled masses, yearning to
breathe free,
The wretched refuse of your teeming
shore
Send these, the homeless,
tempest-tossed to me."*

But who will light, once again, the lamp beside the golden door for the despairing waifs from Vietnam? It has been predicted that one of each two will die by drowning. There is a ray of practical idealism in the darkness: a Hong Kong official has pointed out that it may be possible to turn the Vietnamese refugees "into productive labour to support our economic growth. But we must obtain a commitment from other nations to absorb the human wave..."

Will America respond? Numerous among those who make decisions for her to-day are the upwardly-mobile descendants of the "huddled masses" whom Emma Lazarus apostrophized. Have they forgotten the obligations of their heritage? □

Quauhtemoc, Father Hidalgo and Emiliano Zapata, men who dared confront the established order against impossible odds. The resourcefulness of the Mexican scientist in achieving what he has in the last ten years shows that such a spirit is still alive. Will it suffice for the coming era? □

news and views

In the coming year at least nine major international meetings are being devoted to the peptides present within neural tissue. The first * was a massive affair: for three days, an audience of at least 700 from all over Europe and North America listened to 28 invited speakers. Almost half the speakers devoted their attention to methodology and described how they had used their own particular skills to investigate one or more of these peptides. The others had clearly been instructed to concentrate on one peptide studied with a great variety of techniques, with only one of which they were acknowledged to be expert. By and large these speakers had been selected from the viewpoint that peptides form a new class of neuroactive substances to which certain generalities apply. For instance, peptides may fall into defined groups, each being derived by cleavage from a large precursor molecule. There also seemed to be support for the idea that peptides released from nerve terminals will in general not only mimic the actions of more conventional transmitters such as amino acids, acetylcholine and catecholamines, but that any new unexplained results are suggestive evidence that peptides may mediate their actions on the postsynaptic cell in novel, unconventional ways.

Since much of the work presented has already been published and will in any event be brought together in the proceedings, I shall draw attention here to some of the novel work mentioned only at the meeting and which I feel may prove to constitute the highlights of 1979.

Identification

In the methods section R. Elde (University of Minnesota, Minneapolis) drew attention to difficulties that have delayed the identification of peptide-containing elements at the electron microscopic level. Although he did not discuss methods based on the use of horseradish peroxidase to identify nerve terminals containing immunoreactivity, it is clear that this type of work is still hampered by the compromises needed to preserve the antigenicity of the peptide-containing tissue. Frequently the concentrations of aldehyde fixatives that are tolerated are too low to ensure the integrity of tissue ultrastructure. It therefore seems that although the reaction product in the horseradish peroxidase method is easy to identify at the electron microscopic level, the thin frozen sections which appear ideal for light microscopy are rarely adequate for ultrastructural studies. However, even in these studies, where the reaction product is in the

Peptides in profusion

from John S. Kelly

terminals and in apparent association with synaptic vesicles or the presynaptic membrane, this may simply reflect the sites with which aldehydes react most readily. To overcome some of these difficulties Coulter and Elde (*J. Histochem. Cytochem.* in the press) have developed a method based on ultrathin serial sections cut from epoxy-resin-embedded material and the preparation of alternate sections for conventional immunofluorescence and electron microscopy. Although the resolution of the material is highly dependent on the thickness of the sections, single areas of fluorescence 0.2 μm in diameter can be recognised on photomicrographs enlarged to 8,500 times magnification and then identified on the corresponding electron micrographs. Many of the small fluorescent areas can be identified as synaptic vesicles. Coulter and Elde have yet to explore the possibility that post-embedding staining of serial sections will facilitate the localisation of a range of different peptides in adjacent or even the same ultrastructural element.

LHRH release explains enigma

In his review of the neurophysiological status of peptides, J. L. Barker (National Institute of Neurological and Communicative Disease and Stroke) drew attention to the findings of Jans and Kuffler (*Proc. natn. Acad. Sci. U.S.A.* **76**, 1501; 1979), which suggested that the release of luteinising-hormone releasing hormone (LHRH) from nerve endings in the frog sympathetic ganglion might explain the enigma of the non-cholinergic, non-aminergic 'late slow e.p.s.p.' first described by Nishi and Koketsu (*J. Neurophysiol.* **31**, 109; 1968). This late slow e.p.s.p. lasts for 5 min and is found in both the large B cells and the small C cells. Although substance P, neurotensin, bombesin, somatostatin, thyrotropin-releasing hormone and angiotensin I and II concentrations up to 0.1 mM had no effect, LHRH produced a slow depolarisation of the ganglion incubated in 1 μM dihydro-B-

erythroidine and 0.1 μM atropine. The effect was reversible and the depolarisation evoked by 20 μM amounts occluded the nerve-evoked 'late slow response'. Similar depolarisations were evoked by smaller amounts (0.01 μM) of the more potent LHRH analogues (D-Ala⁶) LHRH and ethylamide (D-Trp⁶, Pro⁹) LHRH. The effect of these analogues was not blocked by the removal of Ca^{2+} from and the addition of Mg^{2+} to the media in concentrations that blocked synaptic transmission through the ganglia. Unpublished work shows both the peptide and nerve-evoked responses to be blocked by LHRH antagonists.

The presence of an LHRH-like substance in the lumbar chain of sympathetic ganglia has also been confirmed by radioimmunoassay using the highly specific antisera developed by Nett and colleagues (*J. clin. endocr. Metab.* **36**, 880; 1973).

Degeneration studies show the LHRH-like material to be confined to the spinal nerves that contain the axons which initiate the late slow e.p.s.p. LHRH-like material is also present in perfusates of the ganglia made with isotonic KCl and the release seems to be calcium dependent. This discovery of LHRH-mediated potentials in the frog sympathetic ganglion draws attention to the possible role of peptides in transmission in the peripheral nervous system. Substance P and enkephalins are now well established as potent transmitters in the guinea pig myenteric plexus and there is still a number of non-cholinergic, non-adrenergic synaptic potentials to be investigated.

Cholecystokinin

Both J.W. Phillis (College of Medicine, Saskatchewan) and S.H. Snyder (Johns Hopkins University School of Medicine, Baltimore) drew attention to their work which shows fragments of cholecystokinin to have potent actions in the frog spinal cord (Phillis & Kirkpatrick *Can J. Physiol. Pharmacol.* in the press) and to be located in specific sites throughout the central nervous system (Innis, Correa, Uhl, Schneider & Snyder *Proc. natn. Acad. Sci. U.S.A.* **76**, 521; 1979). Earlier, Rehfeld and his associates (Larsson & Rehfeld *Brain Res.* **165**, 201; 1979) showed the biologically active octapeptide of cholecystokinin to be located throughout

*A symposium on The Role of Peptides in Neural Function was held on 7-9 May at the National Institutes of Health, Bethesda, Maryland. It was sponsored by the Intramural Program of the National Institute of Neurological and Communicative Disorders and Stroke, and organised by J. L. Barker and T. G. Smith, who will edit the proceedings which will be published by Dekker.

J. S. Kelly is a senior member of the scientific staff of the MRC Neurochemical Pharmacology Unit, Cambridge, appointed Professor of Pharmacology at St. George's Hospital Medical School, London from 1 July 1979.

the central nervous system. Within the spinal cord its localisation in the substantia gelatinosa is reminiscent of the distribution of substance P in fibres thought to be involved in nociception. Furthermore, they have demonstrated similar immunoreactivity throughout the autonomic nerves and ganglia of the gut and have a considerable amount of biochemical evidence to suggest that the neurotransmitter released from these nerves is the terminal tetrapeptide common to cholecystokinin and gastrin (Trp-Met-Asp-Phe-NH₂). On the isolated perfused porcine pancreas, Rehfeld and colleagues have shown the tetrapeptide to be a highly potent and specific releaser of insulin, glucagon, somatostatin and pancreatic polypeptide and suggest that the tetrapeptide may be the neurotransmitter of the nerves which innervate the pancreatic islet cells. Dodd and Kelly (*Proc. Physiol. Soc.*; 1979) have confirmed and extended Phillis's suggestion, that the action of cholecystokinin on central neurones is excitatory, by showing that both the octapeptide and tetrapeptide fragments of cholecystokinin excite rat hippocampal neurones.

Retinal peptides

In his review of somatostatin as a neuroactive peptide, J.B. Martin (Harvard Medical School, Boston), drew attention to his work with Rorstad and Brownstein (*Proc. natn. Acad. Sci. U.S.A.* in the press), in which they demonstrate the presence of radioimmunoassayable and bioassayable somatostatin-like material in acid extracts of the retina by using the release of growth hormone from dispersed rat anterior pituitary cells in culture as their assay. Gel-filtration chromatography confirmed that 96% of the material from the retina could be eluted as a single peak identified by the addition of synthetic somatostatin to the system. Increased levels of somatostatin were found in the retina of rats with hereditary degeneration of the photoreceptor cells and in rats in which transection of the optic nerve one year earlier had led not only to complete atrophy of the optic nerves but also to degeneration of the ganglion cells. The somatostatin therefore seems to originate within the retina. This work is complementary to that of Marshak and colleagues (Marshak, Yamada, Basinger, Walsh & Stell *Invest. Ophthalmol. vis. Sci.*, *ARVO Abstr. No. 25, 85*; 1979), who have used similar techniques to demonstrate the presence of somatostatin-like immunoreactivity in extracts of the frog, rat and goldfish retina. They have extended this work on 10 μ m cryostatic sections of the goldfish retina by using a conventional immunofluorescence method to show immunoreactivity in two types of amacrine cells and one type of ganglion cell. The processes of the cells ramify in the most distal and most proximal sublaminae of the inner plexiform layer.

A more extensive examination of the localisation of distinctive populations of various peptide-containing amacrine cells in the retina by Brecha, Karten and Laverack (*Proc. natn. Acad. Sci. U.S.A.* in the press) was not, however, mentioned at the meeting. Brecha *et al.* have shown enkephalin-like, substance P-like and neurotensin-like immunoreactivity in the avian retina to be localised in separate subpopulations of amacrine cells with processes that arborise in quite different regions of the inner plexiform layer. The distribution of amacrine cells containing enkephalin-like material is quite regular, with the greatest density in the central region where they are spaced at approximately 40- μ m intervals. Enkephalin-like reactivity is also a feature of the amacrine cells of the turtle. Subpopulations of amacrine cells containing substance P-like reactivity are found in the retina of frogs, toads, rats and rabbits.

The presence but not the location of a Met-enkephalin-like material in the avian retina was earlier demonstrated by Humbert, Pradellos, Gros and Dray

(*Neurosci. Lett.* **12**, 259; 1979) using highly specific radioimmunoassays in conjunction with high pressure liquid chromatography and gel filtration. Earlier, other workers reported the presence of thyrotropin-releasing hormone-like immunoreactivity in the rat retina (Schaffer *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 579; 1977) and showed the levels of this material to differ in the light and dark. One hopes that these new studies which show subpopulations of amacrine cells to contain at least five different peptides will go some way to elucidating the function of the 15 different types of amacrine cell which Cajal identified in the retina.

The discovery of a possible LHRH pathway in frog sympathetic ganglia, cholecystokinin pathways in the nerves to the islet cells of the pancreas and possibly the spinal cord and the presence of various peptides in the amacrine cells of the retina surely must lead to the conclusion that the peptides in the brain behave no differently from the other substances already thought to be established as neurotransmitters in the central nervous system. □

Atmospheric arsenic

from Peter Brimblecombe

ARSENIC is toxic. This much at least seems to have been known for a long time. Its environmental chemistry is intriguing, because not only does it occur in various oxidation states, but it is converted to volatile compounds by methylation in reducing sediments in much the same way as mercury. The toxicity and mobility of arsenic have made it the subject of many studies in specific ecosystems, but Walsh, Duce and Fasching have discussed the global cycle of the element in a recent issue of the *Journal of Geophysical Research* (**84**, 1710, 1719; 1979). Their two papers on tropospheric arsenic reflect an increased concern that anthropogenic emissions are significantly altering the distribution of trace elements in the environment.

Man's influence on the levels of airborne arsenic has been a matter of concern since the seventeenth century when John Evelyn wrote hopefully in his diary that the charring of London's coal would remove its 'arsenic malignity'. Salmon and his group at Harwell in a more recent study (*Sci. Total Env.* **9**, 161; 1978) have also implicated coal smoke in the elevation of the arsenic concentrations in the air over rural England. They have examined the long term trends in atmospheric trace elements at Chilton in Oxfordshire, through the use of airborne dust samples collected on filters between

1954 and 1974. The arsenic levels commenced at about 8 ng m⁻³ in the late 1950s and declined to slightly less than 5 ng m⁻³ by the early 1970s. These closely follow the concentrations of smoke in the atmosphere, both in the gradual decrease, and the seasonal pattern of high levels in winter and low values in the summer.

There are good reasons for such a correlation. Arsenic is present in coal at levels of about 25 p.p.m. and almost all of this is released during combustion. Of the toxic elements lead, thallium, cadmium and selenium, only lead is found at levels as high as that of arsenic in fly ash. Like these other low boiling point elements, arsenic concentrates preferentially on the smaller sized particles by vapour transport within the furnace. Goldberg has extended this notion to ambient temperatures, arguing that the relatively high amounts of these elements in the atmosphere might well be explained by evaporation from rocks (*Geochem. J.* **12**, 75; 1978) although in the absence of amplification mechanisms this contribution is probably insignificant.

The concentration of arsenic in rural air over England appears to be a few ng m⁻³, while concentrations determined in remote areas are somewhat lower. (Table 1). The enrichment factors relative to crustal elements range between 23 and 1,940 with a geometric mean of 313. This is largely due to the fact that the arsenic is found associated with sub-micron particles in which the crustal reference

Peter Brimblecombe is in the School of Environmental Sciences, University of East Anglia.

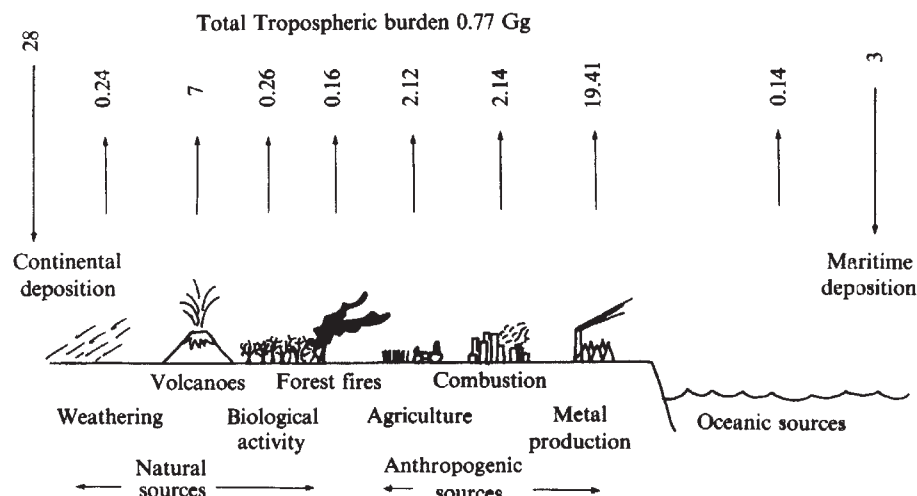


Fig. 1 Global cycle of arsenic (after Walsh *et al.*) Emission and deposition as Gg yr⁻¹ (10⁹ g yr⁻¹).

elements are depleted. The association with the small particle fraction hints at a vapour phase origin.

Walsh and his colleagues were at pains to distinguish between particulate and vapour phase arsenic (that is, < 0.05 µm). With the exception of some unusual events vapour phase arsenic accounted for only a few per cent of the atmospheric concentrations. This is to be expected as compounds with vapour pressures less than 10⁻¹¹ atm are predominantly associated with particulates; the aerobically stable arsenic trioxide has a vapour pressure of only 6 × 10⁻¹² atm, although they note that the ratio of particulate to vapour phase was inversely related to air temperature. In spite of the low concentrations of arsenic in the vapour phase it is important to remember that such low concentrations may reflect a species with a relatively short lifetime, but one which could be of considerable importance in distributing arsenic among the smallest aerosol particles.

The estimation of emission rates on a global scale is difficult because even where measurements are available the particle size is usually unknown. Natural sources are often even more difficult to evaluate because of the extremely low levels of arsenic involved. The largest natural source would appear to be volcanic (Fig. 1), but it is obvious that this could be subject to wide variations. Wind blown dust and particles from forest fires may both make contributions to atmospheric arsenic. The sea is an emitter and here volatilisation of the small amounts of naturally occurring methyl arsenic compounds may be significant. Biological sources account for about 3%, but even subtle changes in the assumptions about

the release of particulates from plants and the methylation of arsenic compounds by bacteria could significantly change the importance of this contribution.

The construction of a global budget for arsenic emphasises the relatively large anthropogenic inputs to the troposphere. The principal use of arsenic is in the manufacture of agrochemicals; arsenic compounds have had a long history of use as insecticides, but during the past 20 years there has been a change from the use of inorganic to organic arsenicals, which

can be applied at a much lower rate. There has also been a marked reduction in its use in insecticides, so that it has become largely restricted to herbicidal compounds. Good evidence for these changes are to be seen in the consumption of arsenic in the United States which halved between 1950 and 1970.

The largest man made contribution to the atmosphere arises from releases incurred in the manufacture of metals, as arsenic is frequently associated with ores. The largest emissions occur in the production of copper. Combustion of fuels is an important source, but coal is really the only fuel of significance, with the incineration of waste making an important contribution. The input from agricultural sources and processes such as cotton ginning is of about the same magnitude as the combustion source. The importance of these anthropogenic sources and the low emission rates from the oceans means that higher levels of arsenic are to be expected in the air over the northern hemisphere. The rates of input seem in reasonable agreement with the measured rates of removal. The tropospheric residence time determined from these rates and the atmospheric arsenic burden suggest a residence time of about 9 days, which is about that which would be expected for a tropospheric aerosol. □

Anomalous H-2 antigens on tumour cells

from Kirsten Fischer Lindahl

FOUR years ago the idea was discussed (*News and Views* 254, 653; 256, 7; 1975) that some tumour-specific transplantation antigens (TSTA) were anomalous forms of the major histocompatibility antigens (H-2 in the mouse) found on normal cells of the individual in which the tumour arose. According to this view, the anomalous H-2 antigens would function as fire-alarms, signalling the transformation of a cell to the immune surveillance system.

A recent meeting* offered an overview of the evidence accumulated since then for the presence of anomalous H-2 antigens on tumour cells. The meeting confirmed published evidence that many tumours lose antigens: a heterozygous lymphoma cell line, LDHB, does so spontaneously at a very high rate in culture (B. Holtkamp, University of Cologne). On the other hand, tumours may gain H-2-like antigens. Some mouse tumour cells express H-2-like antigens which in normal spleen cells from the same mouse strain are detectable only in lysates and even then in lower amounts than in the tumour cells. This is the case for the DBA/2 lymphoma, SL2 (P. Robinson,

Deutsche Krebsforschungszentrum, Heidelberg) and a retinal cell sarcoma (B. Bonavida (University of California, Los Angeles).

A spontaneous AKR (H-2^k) leukaemia, K36, apparently loses expression of antigens controlled by the H-2K^k locus, as detected serologically or by T killer cells specific for the H-2K^k locus. Furthermore, K36 appeared to express two different allelic products of the H-2D locus: the expected H-2D^k antigen and new H-2D^d-like specificities. W. Schmidt (London Hospital Medical College) showed preliminary peptide maps of the H-2D^d-like antigen precipitated from the K36 tumour cells. These maps were very complex, presumably because of coprecipitated viral antigens; but they seemed to contain all the peaks found in a peptide map of H-2D^d from normal cells.

The most persuasive evidence for anomalous specificities on tumour cells came from F. Garrido (Facultad de Medicina, Cordoba) and V. Schirmacher (Deutsche Krebsforschungszentrum, Heidelberg) who have studied MCG4, a methylcholanthrene-induced BALB/c (H-2^d) tumour which has been converted to growth as ascites. The expected H-2^d antigens were expressed only weakly on the tumour cells. Normal BALB/c mice

Table 1 Concentrations of atmospheric arsenic

	(ng m ⁻³)
Northern Canada	0.3
Sahelian dust plume	1.2
Volcanic plume	2.2 and 5.5
Antarctica	0.019
Bolivian mountains	1.4
Oceanic air	< 1.0

*A meeting on 'H-2 Antigens: Genetic Control and Expression on Normal, Tumour and Virally Infected Cells' was held at the London Hospital Medical College on 30 April-3 May. The meeting was sponsored by the European Molecular Biology Organisation and the Cancer Research Campaign of Great Britain.

responded to the tumour cells with formation of cytotoxic T cells and of antibodies; the latter reacted with cells of almost all mouse strains (except H-2^d strains). Although the MCG4 cells did not express the private serological specificities of H-2^k, they did react with four different monoclonal BALB/c anti-H-2K^k antibodies. Furthermore T killer cells which specifically attacked MCG4 cells also recognised H-2K^k and H-2D^k. MCG4 may also carry some H-2^b-like specificities.

Susceptibility of the tumour cells to BALB/c killer cells raised against foreign H-2 antigens does not prove the presence of a particular gene product, because such killer cells are known to be very cross-reactive (more evidence on this point was presented by C. Neauport-Sautes, IRSC, Villejuif). More dramatic was the demonstration that Sendai virus-infected MCG4 tumour cells, but not virus-infected BALB/c lymphoblasts are susceptible to H-2K^k-restricted T killer cells from B10.A(4R) mice specific for Sendai virus. Because H-2 restricted killer cells are generally extremely specific, this was taken as evidence that MCG4 expressed the restriction specificity of H-2K^k. It must, however, be noted that two cases were presented at the meeting where precisely H-2K^k restricted T killer cells could recognise other restriction elements: Elizabeth Simpson (Clinical Research Centre, Harrow), showed that male-specific killers also reacted with H-2D^k male target cells, and W. Haas (Basel Institute for Immunology) has shown that even cloned killer cells specific for the hapten SP, killed hapten-coupled cells carrying H-2D^k, H-2D^b or H-2^r antigens.

Are the new H-2-like specificities transplantation antigens? This seems to be the case for MCG4 which grows better in (BALB/c × H-2^k)F₁ mice than in BALB/c, the strain of origin. Immunisation of BALB/c mice with BALB.K (H-2^k) cells increases their resistance to C-1, another BALB/c tumour found by G. Parmiani and G. Invernizzi (Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan) to express H-2K^k serological specificities. That this tumour was rejected equally efficiently by several (BALB/c × H-2^k)F₁ hybrid mice and by BALB/c may mean that the tumour cells have other TSTA in addition to the H-2K^k-like ones. H-2-like TSTA is one possible explanation why M. Bortin (Mount Sinai Medical Center, Milwaukee) could induce resistance in AKR mice to syngeneic leukaemia by immunisation with pooled allogeneic cells, carrying many different foreign H-2 antigens. On the other hand, E. Lennox, (MRC Laboratory of Molecular biology, Cambridge) could biochemically separate the TSTA of a couple of methylcholanthrene-induced tumours from their H-2

antigens by their different affinities for lectins.

In spite of the many reports of new H-2-like antigens on tumour cells, other laboratories study tumours without finding such evidence, and the devil was not short of advocates at the meeting. C-1 is the only tumour so far which has been shown to have the same isoenzyme markers as the presumed strain of origin. But neither this test, nor an analysis of frozen samples of the very mouse from which the tumour was taken would exclude a mix-up with another cell line from an H-2 congenic strain during the many passages in mice or in culture.

Special caution is needed in the interpretation of serological data. Are the antigens detected on tumour cells with anti-H-2 sera also structurally H-2-like, and are the antigens detected by syngeneic antitumour sera on normal mouse cells from other strains H-2-like, not only in strain distribution, but also in tissue distribution and ontogeny? P. Ivanyi (Central Laboratory of the Netherlands Blood Transfusion Service, Amsterdam) presented a cautionary example of an anti-H-2^b serum raised in a virus-free B10.A(H-2^a) line against C57BL/10 cells from a line carrying a milk-transmitted virus. Although these antibodies were completely removed by absorption with virus-free C57BL/10 cells, and thus were H-2^b specific, they also reacted with syngeneic B10.A cells from a line carrying the same virus. This antigen, which was dependent on the milk-transmitted virus, did not appear on spleen cells until day 17 after birth.

In no case has it yet been demonstrated that the new H-2-like specificities are actually encoded by chromosome 17 (as are the known H-2 specificities), but assuming this is so, how do they arise? It has been proposed that "the serologically detected allelic differences [of H-2] are actually the products of different, closely linked genes, and the genetic polymorphism is for the control of whichever of the genes is expressed" (Bodmer *Transplant. Proc.* 5, 1471; 1973). The day now seems close when this hypothesis will be tested directly by isolation of the genes of the H-2 complex and sequencing of the DNA. According to this model, new H-2-like specificities on tumour cells could arise by derepression of the corresponding genes. The current argument against derepression (and incidentally against a mix-up of cell lines) is that the anomalous antigens had only some, not all, of the specificities of known H-2 haplotypes: an H-2^d tumour might cross-react with H-2^k at the T cell level but still lack the private serological specificities of H-2^k.

Mutation seems a likely cause of new specificities. In normal mice mutations can occur in H-2K and H-2D fairly frequently (4×10^{-5} per locus per gamete). Such mutants have been detected by their ability to cause skin graft rejection. Studies in

vitro on cytotoxic T cell reactions between mutant and wild type cells (K. Melief, Netherlands Cancer Institute, Amsterdam) showed that such mutations give rise to multiple new specificities, some of which cross-react with known foreign H-2 antigens (Nabholz *et al. Immunogenetics*, 1, 457; 1975). J. Martin (National Cancer Institute, Bethesda) showed that a transplacentally-induced lung tumour from a C3HfB/HeN mouse — this strain apparently carries an H-2K^k mutation — expresses wild type H-2K^k antigens, as if it were a revertant, and grows progressively in normal C3H/HeN mice.

Considering the strong reactions elicited by mutant cells, the report by S. Nathenson (Albert Einstein College of Medicine, New York) on the amino acid sequence analysis of eight H-2K^b mutants was indeed surprising. Seven of these had only a single amino acid substitution, and one had two, five residues apart. Furthermore, the changes were all conservative, such as methionine to leucine. Nathenson presented a model in which the H-2K molecule can be likened to a bow knot, the N-terminal end sticking out from the cell surface, the C-terminal end traversing the cell membrane, and two domains held by cystine bridges forming the loops. All the mutations were clustered around the two cysteines of the first domains, suggesting that this is the site of prime importance for T-cell recognition (some of the mutations even lead to changed restriction specificity for virus-specific killer cells). It is not known whether the many amino acid differences between alleles at the N-terminal end play a part serologically.

The reason for the strong T cell reactivity to H-2 mutants may be that T cells during their development are selected for their ability to see minor alterations of self. Presumably somatic mutants that express altered H-2 antigens arise all the time and are normally eliminated by the immune system. It seems structurally almost unavoidable that altered self H-2 antigens would share specificity with one or more foreign H-2 antigens, and priming against such altered self determinants might account for the high frequency of alloreactive T cells in a normal mouse. □

Glueballs without gullibility

from Probir Roy

A glueball is a newly proposed form of neutral, unstable, quarkless hadronic matter and as such is currently engaging the attention of high energy particle physicists. Hadrons, the strongly interacting elementary particles, are assumed to be composed of pointlike (that is structureless) permanently confined constituents, the quarks. Apart from its spin of half, and fractional charges $\frac{2}{3}$ and $-\frac{1}{3}$

Kirsten Fischer Lindahl is a member of the Basel Institute for Immunology, Basel, Switzerland.

in units of the proton charge, each quark carries three kinds of a quantum number called colour. Colour is necessary to satisfy the antisymmetrical Fermi-Dirac statistics property of quarks in describing baryons as three-quark composites, the three quarks being in a colour-antisymmetrical state. Colour is also carried in eight different kinds by massless boson quanta (of spin one) called gluons. These are responsible for keeping the quarks 'glued' together in a hadron and are themselves also confined. One special property of colour forces mediated by gluons is the self-coupling of three gluons carrying different colours. As a result, the colour forces become weaker and weaker at shorter and shorter distances. This phenomenon is poetically described as 'asymptotic freedom' and seems to have been observed experimentally (see *News and Views* 277, 349; 1979). These forces are believed to become stronger at greater distances. The literary acronym for this behaviour is 'infrared slavery' and this is what is supposed to be responsible for colour confinement. According to this view all coloured objects (such as quarks and gluons) should be confined and only colourless hadrons should be observable.

Given the self-coupling of gluons, one can expect to see as real, unstable particles of zero or integral spin, colourless composites made out of gluons alone. These are the hypothetical (at present) glueballs. A glueball may be visualised as a composite of two or three gluons, in analogy to the quark-antiquark picture of a meson or the three-quark picture of a baryon. Several glueballs are likely to exist at different masses. It has been argued (*Nucl. Phys. B* 130, 328; 1977) that, owing to the asymptotic freedom of the colour forces, the decay of the lowest mass glueball into the usual hadrons ought to be somewhat inhibited. Consequently, it is expected to be more stable than a typical meson of the same mass. It should have a lifetime of the order of 10^{-21} rather than of the order of 10^{-23} as is usual for mesons. Higher mass glueballs will of course be generally more unstable.

The existence of glueballs was first proposed by Fritzsche and Gell-Mann in 1972. However, they remained an idle curiosity until an intriguing suggestion by Freund and Nambu (*Phys. Rev. Lett.* 34, 1645; 1975) linked them to an important feature of high energy hadron-hadron collisions. The total cross-section for such collisions depends very weakly on the energy - a behaviour which had for many years defied any real understanding in terms of the otherwise successful Regge picture of exchanges of Regge trajectories on which hadrons lie. Freund and Nambu could provide a credible explanation if the dominant exchanges are those of new Regge trajectories on which real glueballs

Incest re-assessed

In his advisory article on incest (*News and Views* 279, 192; 1979), May reinforces his theoretical calculations on the deleterious effects of parent-offspring and brother-sister matings by reference to a Czechoslovakian study reported by E.O. Wilson. As presented, the study provides overwhelming evidence to support May's hypothesis; however a closer examination of the original data must raise a number of doubts as to the validity of the control group employed.

In her study (*Hum. Hered.* 21, 108; 1971) Seemanova reported that the mean and the modal maternal ages of the non-incestuous control group at childbirth were 24.9 and 21 years while those of the father-daughter matings were 18.9 and 16 years and the brother-sister matings were 19.9 and 14 years. Since the Czech data referred to births from 1933 to 1970, the low modal ages of the mothers in the two incestuous groupings are particularly significant when viewed against the secular decline in the age of menarche during that period (Tanner *Growth at Adolescence* Blackwell, Oxford, 1962) and the high incidence of chromosomal anomalies and congenital malformations associated with pregnancies

close to menarche (Carr *Adv. Hum. Genet.* 2, 201; 1971).

A second area of doubt concerns the imbalance between the biological fitness of the parents in the incestuous and the non-incestuous matings. Of the 141 incestuous mothers, 20 were mentally retarded (of whom two additionally were deaf-mutes, two had congenital syphilis and two were epileptics), a further two were deaf-mutes and three were schizophrenic. Among the 46 control mothers, a subgroup of the incestuous mothers, only two were mentally subnormal (one additionally being a deaf-mute) and two others were deaf-mutes. Similarly, of the 138 fathers in the incestuous matings 8 were mentally subnormal, 13 were chronic alcoholics, two had syphilis and four had committed suicide. In the non-incestuous grouping of 52 fathers, none were mentally subnormal, there were two chronic alcoholics and one case of polydactyly.

When considered in conjunction with the specific adverse social factors accompanying incestuous pregnancies, the conclusions on incest drawn on the basis of this particular study appear rather less convincing.

Chelsea College,
London.

A.H. BITTLES

lie. They required glueballs to exist with spins two, one and zero with masses around 1.5 GeV.

The very existence of glueballs - let alone their masses - is still a matter of considerable theoretical controversy. It was proved by Coleman (*Comm. Math. Phys.* 55, 113; 1977) that, if the colour forces are described by the laws of classical physics, glueballs cannot exist. In a classical world a glueball would show up as a concentrated colourless gluon-made packet of finite energy which does not spread. Coleman has shown however that all classical packet-like solutions of this theory radiate away their energy very fast. Of course, the nonexistence of classical glueballs says nothing about the existence of quantum glueballs. However, it suggests that a glueball would be a purely quantum occurrence. The question of the existence of a glueball in a quantum field theory is a difficult one but has nonetheless received some attention. Arguments have been given (though not universally accepted) for the existence of such objects in a quantum theory of colour forces in lower dimensions, but none has yet been found for the (3+1) dimensional space-time in which we live.

Instead of pursuing such a general approach, model-builders have considered the glueball question in specific dynamical models. The bag model, which has been developed particularly by the MIT group, is one such scheme. In this, each hadron is

considered as a bag in which quarks and gluons are kept confined by chosen boundary conditions. The bag generates volume pressure and/or surface tension to produce confinement. Gluon wavefunctions have been treated by analogy to the transverse electric and magnetic modes of electromagnetic fields confined within a spherical cavity. This approach leads to the prediction of glueballs with spins zero and two and with masses slightly less than 1 GeV.

Another set of models has been pursued by lattice theorists, led by Wilson. They quantise space-time and consider the theory of colour forces on a four-dimensional Euclidean (that is, $x_1, x_2, x_3, x_4 = it$) lattice with nearly vanishing lattice spacings. Quarks and antiquarks, instead of being continuous fields, are attached to lattice sites and gluons identified with the links between such sites. A quarkless glueball then is a closed box of links with no loose ends. Such objects have been shown to exist with spins zero, one and two and their masses calculated by Padé approximant methods to be around 1.5 GeV.

Glueballs have been looked for experimentally although they are generally very difficult to produce in hadronic collisions. The gluons by and large play a passive role in these reactions. However, they do play a pivotal part in the decay of heavy quark-antiquark bound states ('quarkonia') produced by electron-positron annihilation at high energies: for

example, the J/ψ at 3.1 GeV and the ψ at 9.4 GeV. In these bound states the heavy quark-antiquark pair can decay hadronically only by annihilating into three gluons in much the same way that positronium disintegrates into three photons. The gluons of course have to evolve into colourless hadrons later. The glueball may lurk among such hadrons. One interesting decay mode is when J/ψ goes into an energetic photon plus hadrons. Here the heavy quark-antiquark pair in the J/ψ annihilate into two gluons and a photon. The two gluons could resonate into a glueball in which case the photon will be highly monochromatic. Experiments done by the PLUTO group at the German storage ring DORIS have yielded good quality data for precisely this situation. They rule out the existence of any glueball up to a mass of 1.2 GeV, thereby demolishing the lighter than 1 GeV glueball of the MIT bag model.

Turning to the much heavier ψ , the three gluons from its decay are expected to generate jet-like hadronic fragments. Normal electron-positron annihilation into hadrons by way of a quark-antiquark pair above 6 GeV produces two symmetrical jets opposite each other. This

jet structure survives the final 'colour-washing' that creates only colourless hadrons out of the quark and the antiquark. As each of the gluons from ψ decay will on average carry an energy of nearly 3 GeV, gluon jets can be expected there. The three gluons should emerge symmetrically with 120° angles between one another, and there is some preliminary evidence for such events from PLUTO (CERN *Courier* 19, 108; 1979). Another interesting possible configuration is one in which an energetic gluon dashes off to one side leaving two less energetic collinear ones opposite. This will lead to two jets opposite each other that are asymmetrical. According to a recent suggestion (Roy & Walsh *Phys. Lett.* 78B, 62; 1978), the energetic gluon can now easily induce the creation of a gluon pair from the vacuum and capture one of its members to form a glueball. The latter should show up as a prominent, not very unstable resonance in one arm of the two jets and should disappear off the ψ mass. Quantitative estimates, albeit hand-waving, have been made and look extremely favourable for the production and detection of glueballs in this manner, if they exist at all. $\square$

beneficial in shock cases. High molecular weight dextran has anticoagulant activity and it can be used for this purpose after surgery. Low molecular weight dextran is antigenic but there is no evidence for this with clinically-used dextran, although allergic reactions occasionally occur. Various dextrans in the molecular weight range of 40,000–110,000 are used depending on the balance of effect desired.

The Chinese work is from the Institute of Organic Chemistry in Shanghai, one of the three big polymer research groups in China. They describe the production of sodium carboxymethyl amylose by carboxymethylation of amylose from corn starch (*Acta Chimica Sinica* 36, 49 (1978)). The molecular weight of the product was characterised by ultracentrifugation, solution viscometry, fractional precipitation and osmotic pressure. Using ^{14}C -labelled carboxymethylation followed by hydrolysis and paper chromatography the reaction was shown to occur primarily on the 2-oxygen. The degradation rate of the polymer in blood decreases with increasing carboxymethylation. A 5% solution of the polymer (molecular weight 60,000, 0.4 carboxyl methyl groups per saccharide) in 0.9% saline is now being tested in animals. It maintains blood volume for 6 h, is 90% lost in 24 h and is said to lack the immunological reactions of dextran. A possible snag, however, is that similar charged polymers are reported to enhance clumping (see Ricketts *op.cit.*)

This work is interesting in the Chinese context for a number of reasons. It is a piece of careful, thorough polymer science. It describes the development of a new material from an available resource to replace the complex fermentation process needed for dextran. The work is original, whereas much of polymer science in China has been devoted to reproducing Western materials from patents. Also the work was done between 1961 and 1964 and has waited 15 years to be published. It shows that a relatively modest project can produce something adapted to local needs.

Most Western interest in this area seems to be in the more dramatic problem of an artificial blood substitute capable of carrying oxygen, particularly for organ storage. Geyer (*Drug Design* VII (ed. Ariens) Academic Press, 1976), has reviewed these with particular regard to emulsions of perfluorochemicals. With these he was able to replace completely the blood of rats so that they survived in 100% oxygen for 10 days until their red cells regenerated and they could be returned to air. Interestingly, these bloodless rats could also survive in 10% carbon monoxide. Cell-free haemoglobin solutions have also been used for blood replacement but they are degraded and must be continually transfused. There is obviously potential here for an oxygen-chelating polymer but a suitable molecule does not yet seem to be available $\square$

Polymers for blood replacement

from Paul Calvert

SYNTHETIC blood volume expanders are sometimes used in cases of extensive bleeding or burns where much plasma has been lost, or in cases of shock, where contraction of the capillaries increases blood pressure so that water is lost to the tissues, the blood thickens and circulation ceases. Restoration of normal volume can be achieved by natural plasma, solutions of plasma proteins or by solutions of polymers such as dextran. Where blood or plasma are readily available the main reason for using a synthetic expander is to avoid the risk of hepatitis. On the other hand, in countries where blood supplies and storage and separation facilities are more limited, synthetic substitutes should be more important. So, recently published Chinese work on a volume expander derived from corn starch is of interest as an application of science to the needs of a developing country.

Blood has such diverse functions that it is difficult to imagine a completely satisfactory substitute. The role of a volume expander is simply to maintain the correct water balance between blood and the tissues and to allow continued transport of nutrients and gases. The water balance is determined by the osmotic pressure between blood and tissues, and the hydrostatic pressure difference. Lost volume can be restored by addition of an isotonic electrolyte solution such as Ringer's but this does not give the correct

osmotic pressure balance as the electrolytes pass readily across membranes into the tissues. It is necessary also to restore the colloidal osmotic (oncotic) pressure normally provided by serum albumin which cannot cross the membrane and so balances the osmotic pressure of the macromolecular cell contents. In practice it does not seem to be agreed whether polymeric (or 'colloidal') solutions work better than salt solutions but they are certainly better in principle (see Pennell, in *Bibl. Haemat.* 29, 883; 1968).

Solutions of a range of water-soluble polymers have been tried as volume expanders. The chief requirements are that the polymer is of sufficiently high molecular weight to prevent its loss through the kidneys, that it resists degradation and causes no harmful reactions. Dextran is chiefly used but hydroxyethyl starch has had successful clinical trials and polyvinyl pyrrolidone has been tested (Ricketts, *Brit. J. Anaesth.* 45, 958; 1973). Dextran has been used for about 30 years and its side effects are well studied (Gruber & Messmer, *Prog. Surg.* 15, 49; 1977); Gronwall *et al.*, *Bibl. Haemat.* 29, 874; 1968). High molecular weight dextrans cause clumping of red cells (rouleaux formation) though, interestingly from the colloid science viewpoint, molecules of low molecular weight lower even the natural degree of clumping. Reduced clumping leads to lower blood viscosity, promotes blood flow and exchange of nutrients with tissues and so is

Paul Calvert is a Lecturer in the School of Molecular Sciences, University of Sussex.

review article

Hormonal regulation of peptide receptors and target cell responses

K. J. Catt, J. P. Harwood, G. Aguilera & M. L. Dufau

Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205

Regulation of plasma membrane receptors for peptide hormones by the prevailing ligand concentration often causes altered target cell function. Receptor number is determined by hormone-induced changes in membrane conformation, irreversible ligand binding, and processing of ligand-receptor complexes during hormone action.

THE ability of target cells to respond to changes in ambient ligand concentration by regulating the number and/or affinity of their surface receptors is now so widely documented in hormone-regulated tissues that the absence of receptor regulation is now considered an unusual finding. Self-regulation of membrane receptors, originally observed with surface antigens, has been demonstrated in cells exposed to ligands including neurotransmitters, peptides, protein and glycoprotein hormones, and surface-modulating factors such as lectins and immunoglobulins¹. Desensitisation of cellular responses by hormonal ligands has been observed in certain target cells, and in some cases has been correlated with loss of receptor sites—'down-regulation'. The mechanism of receptor regulation by homologous ligands probably depends on the ability of peptide hormone receptors to behave as mobile entities within the plasma membrane. The mobility of hormone receptors is evident during their interactions with membrane effector proteins such as adenylate cyclase, and in ligand-induced patching and capping of receptors². Investigations of the fate of receptors during ligand-induced regulation have drawn attention to the ability of peptide hormones to enter their target cells, in contrast to the previous view that internalisation was a minor or negligible aspect of protein hormone action³.

The effects of hormone stimulation on target cells include a complex series of events that not only evoke the characteristic cellular response, but also regulate the components of the activation pathway and modify the response to subsequent hormone stimulation. This article reviews recent work on the regulation of hormone receptors, with emphasis on the mechanisms and consequences of receptor regulation and desensitisation in target cells for insulin, catecholamines, angiotensin II and gonadotropins.

General aspects of receptor regulation

The concept of intrinsic membrane proteins diffusing laterally within the lipid bilayer⁴ led to the notion that the mobility of receptors within the membrane was responsible for features of peptide hormone action such as the activation of common membrane responses by multiple independent ligands⁵, desensitisation, negative cooperativity, and the dependence of receptor affinity on the presence of membrane effector systems⁶. One prediction of the hypothesis of mobile receptors is that the receptors should be physically independent of adenylate cyclase in systems where the response is effected via adenylate cyclase. That prediction is borne out by the resolution of the two

activities in detergent-solubilised membranes from cells bearing receptors for glucagon⁷, luteinising hormone (LH)⁸, or catecholamines⁹, by the coupling of receptors from one cell type with the heterologous adenylate cyclase of another cell type when the two are fused^{10,11}, and by the functional transfer of solubilised ovarian LH receptors to isolated adrenal cells¹². This evidence suggests that there is a universal mechanism of coupling between receptors and adenylate cyclase but characterisation of solubilised receptors^{13–17} has not yet revealed the molecular basis of the supposed coupling.

Edelman² has suggested that cell growth, movement and recognition are coordinated by a supramolecular complex of cell-surface receptors and submembranous fibrillar structures, by which changes (surface modulation) in receptor conformation, mobility and distribution lead to changes in the associated cytoplasmic components. These structural interactions are probably responsible for the aggregation (clustering, patching and capping) of receptors that is induced by cross-linking agents such as divalent antibodies and multivalent lectins.

The clustering of receptors into surface aggregates is an important prelude to internalisation of hormone-receptor complexes, as discussed below but may also be important in the acute phase of hormone action. Thus, bivalent antibodies to the insulin receptor can both bind to receptors and exert insulin-like actions in isolated adipocytes¹⁸. In contrast, monovalent Fab' antibody fragments, although binding to the receptor, do not evoke a biological response. Addition of anti-F(ab')₂ antiserum cross-linked the Fab' receptor complexes and restored the insulin-like action of the antibody; also, anti-insulin antibodies enhanced the biological activity of low concentrations of insulin. These effects were independent of microfilaments and microtubules, and indicated that receptor cross-linking or aggregation is important in the insulin-like effects of anti-receptor antibodies and perhaps in the actions of insulin itself. This proposal is consistent with findings that insulin receptors are mobile¹⁹ and can form clusters²⁰ in the cell membrane, and with the ability of lectins²¹ to exert insulin-like effects.

Many peptide hormone receptors are regulated by the homologous hormone, and sometimes by other hormones. Increased hormone concentrations usually cause a decrease in the respective receptors, as demonstrated originally by Roth and co-workers for the insulin receptor of liver^{22,23} and cultured lymphocytes²⁴. Such down-regulation of insulin receptors occurs in human obesity²⁵ and in genetic or induced obesity in rodents²⁶, and probably reflects the action of excess circulating

insulin to decrease insulin receptors. Such receptor loss seems to be a general consequence of the raised blood insulin levels associated with insulin-resistant states. The converse effect, of increased insulin receptors in states of reduced insulin secretion, has been observed in the liver of diabetic hamsters and rats^{27,28}. Other receptors down-regulated by the homologous hormones include those for growth hormone, thyrotropin-releasing hormone, catecholamines, gonadotropins and glucagon²⁹⁻³⁵. Certain peptide receptors, such as those for prolactin³⁴ and angiotensin II³⁵, may increase in number after exposure to elevated hormone concentrations, but the more common response is loss of receptors from the cell surface. Some receptors, notably those for insulin, may exhibit negative cooperativity on exposure to increased ligand concentrations. This represents an additional form of receptor regulation, in which partial occupancy leads to decreased affinity of the residual sites, with acute reduction of target cell sensitivity in the presence of increased insulin concentrations³⁶. Hormone-induced receptor loss has sometimes been found to be dependent on protein synthesis^{30,36} and sometimes not^{29,31}, but the return of peptide receptors seems to require new protein formation²⁹. The details of the processes leading to receptor depletion and replenishment are still not clear.

An important factor in receptor regulation is the incompletely reversible nature of the complex formed after the initial phase of hormone binding to the receptor. Analysis of hormone-receptor interactions has traditionally involved methods based on the assumption that the complex is reversible, but kinetic studies have frequently shown the hormone-receptor complex to have an initial rapid phase of dissociation, followed by a prolonged phase of slow and incomplete reversal³⁷. This suggests that the initial interaction with hormone leads to a conformational change in the receptor that results in tighter binding of the ligand. One view is that the higher-affinity binding state is brought about by interaction of occupied receptors with adenylate cyclase³⁸, but similar changes are evident in receptors considered not to operate through adenylate cyclase, such as insulin³⁹ and angiotensin II⁴⁰. In any case, prolonged occupancy by undissociated ligand is always a potential cause of functional receptor loss, and should be evaluated during studies of receptor regulation. For this purpose, uptake and release of labelled ligand and direct assay of occupied and free sites should be performed, since multiple washing steps and unchanged basal adenylate activity do not exclude residual occupancy by the agonist ligand. As well as causing apparent receptor loss by masking free binding sites, continued occupancy by ligand favours the probability that hormone-receptor complexes will be degraded or otherwise processed and destroyed. This could involve occlusion within the cell membrane or shedding from the cell surface, but accumulating evidence favours a process of endocytosis and subsequent lysosomal breakdown of the internalised hormone-receptor complexes.

Effects of receptor regulation on cell responses

Implicit in the interpretation of receptor regulation has been the expectation that end-organ responsiveness will be altered by changes in receptor concentration. If no other changes occurred, a reduction in membrane receptors would be expected to decrease target-cell sensitivity in tissues containing abundant 'spare' receptors, and to decrease the magnitude of the target-cell response when receptor concentration became the limiting factor in the ability to elicit a hormonal response. The general function of receptor regulation as a mechanism to decrease target-cell sensitivity in the face of high hormone concentrations could have certain biological advantages. An obvious example is blood glucose homeostasis, in which decreased insulin receptors and reduced sensitivity of peripheral tissues to insulin would blunt the effect of hyperinsulinaemia on circulating glucose concentration. Receptor regulation could also minimise or buffer the cardiovascular responses to excessive levels of angio-

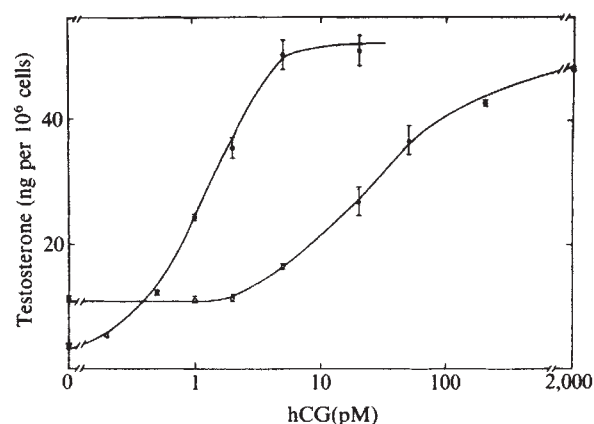


Fig. 1 Decreased sensitivity of testosterone responses of desensitised Leydig cells to gonadotropin stimulation *in vitro*. Cells were prepared from testes of adult male cats treated 48 h earlier with 10 μ g hCG by subcutaneous injection (○), or from untreated controls (●). The loss of LH receptors causes a marked increase in the ED₅₀ for hCG-stimulated testosterone production.

tensin II and catecholamines. This could account for the marked resistance to angiotensin II that occurs in many clinical states of increased renin secretion. However, the biological function of receptor regulation is less evident for other stimulators, such as growth hormone and gonadotropins, which exert more long-term biological effects on their target cells.

The effects of receptor regulation on cellular responses to hormones have been studied in several tissues, particularly target cells in which hormone action is mediated by the adenylate cyclase-protein kinase system. Intact cells are particularly useful for such studies, because hormone binding and cyclic AMP production can be correlated with the ultimate cell response⁴¹. Also, the cyclic AMP produced during hormonal stimulation of isolated cells frequently accumulates in the medium, providing a sensitive index of receptor activation. An important distinction to be made in the study of such hormone-treated tissues is the difference between acute desensitisation (usually of adenylate cyclase) without receptor loss, and the development of true receptor loss, which is a delayed and more slowly reversible consequence of hormone-receptor interaction.

Analysis of the level at which regulation of surface receptors causes altered metabolic responses depends on the ability to measure changes in the hormone-activated pathway that is triggered by receptor binding. For several peptide hormones, such as insulin, growth hormone and prolactin, the immediate mechanism of action on target cells is still unknown. Thus, although insulin indirectly modulates several membrane enzymes, including adenylate cyclase, phosphodiesterase and ATP-ase, the relationship of these effects to its actions on membrane transport systems and anabolic responses remains unclear⁴². Where early events in hormone action have not been identified, the effect of receptor regulation on cell responses can be examined by measuring changes in transport systems and biosynthetic processes. This approach has been used to analyse the effects of insulin receptor regulation in adipocytes or hepatocytes, on glucose or amino acid transport and lipid synthesis^{43,44}.

The consequences of receptor regulation on target cell function have been extensively studied in steroidogenic cells of the testis and ovary, which respond to trophic hormones with specific steroid secretion via an adenylate cyclase-dependent mechanism. The Leydig cell of the testis and the luteal cell of the ovary possess high-affinity LH receptors that are readily measured with ¹²⁵I-labelled human chorionic gonadotropin (hCG) and are coupled to steroidogenesis through the adenylate cyclase-cyclic AMP-protein kinase pathway^{41,45}. In these cells it is possible to analyse several levels of response, from receptor

occupancy through cyclic AMP production and kinase activation, to stimulation of pregnenolone production and subsequent biosynthetic steps leading to progesterone and testosterone production. Analysis of these processes in enzyme-dispersed cells during hormone stimulation permits evaluation of the changes induced by gonadotropin levels similar to those regulating cell responses *in vivo*. When cells are prepared after desensitising doses of gonadotropin, marked changes in LH receptor content and metabolic responses to hormone stimulation are evident *in vitro*. As described below, such desensitised cells provide evidence for several consequences of receptor regulation induced by hormone treatment in the intact target tissue.

Regulation of β -adrenergic receptors and adenylate cyclase

The actions of catecholamines on receptors and adenylate cyclase have been analysed in avian and amphibian erythrocytes, and in cultured cells bearing β -adrenergic receptors. The ultimate cellular actions of catecholamines in most of these test systems are not yet known, although an association with ion transport has been described in the avian erythrocyte⁴⁶.

Measurement of β -adrenergic receptors has been performed by binding assays with labelled antagonists, either ¹²⁵I-hydroxybenzylpindolol⁴⁷ or ³H-dihydroalprenolol (DHA)⁴⁸. In frog erythrocytes, binding studies with DHA have shown available β -adrenergic receptors and adenylate cyclase responses to be reduced by previous exposure to catecholamines^{31,48}. In contrast, the β -receptors in turkey erythrocytes do not exhibit altered binding properties on exposure to adrenergic stimuli (G. D. Aurbach, personal communication). The desensitising action of catecholamines in frog cells can be blocked or reversed by the β -adrenergic antagonist propranolol, which does not itself cause desensitisation. These findings indicate that desensitisation or tolerance to catecholamines is caused by a reduction in the number of functional β -adrenergic receptors. It is likely that chronic occupancy by agonist rather than an active process of receptor loss is responsible for the decreased receptor number, as the ligand-induced changes in receptor number and adenylate cyclase were not altered by treatment with cycloheximide to inhibit protein synthesis⁴⁹. Desensitisation was also observed in erythrocyte membranes exposed to β -adrenergic agonists *in vitro*, but was not produced by antagonists such as propranolol, and could be reversed by guanyl nucleotides⁵⁰. These findings suggest that receptor binding of agonist is followed by coupling of the complex to adenylate cyclase, a process accompanied by conformational changes that initially activate the enzyme and subsequently produce the refractory state known as desensitisation. A requirement for such coupling would account for the inability of antagonists to induce desensitisation, and of guanyl nucleotides to reverse desensitisation by enhancing dissociation of the receptor-bound agonist.

Subsequent studies on the interaction of erythrocyte receptors with a labelled β -adrenergic agonist, ³H-hydroxybenzyl-isoprenaline (HBI), revealed that dissociation of bound agonist is much slower than that of antagonists such as ³H-DHA, and is significantly accelerated by guanyl nucleotides⁵¹. The persistence of agonist binding could be responsible for desensitisation of β -adrenergic receptors in cell membranes exposed to agonists *in vitro*, and for the apparent loss of receptors measured with labelled antagonists. Formation of the high-affinity complex between β -receptor agonists and adenylate cyclase-coupled receptors is dependent on magnesium, which increases receptor binding affinity for agonists but not for antagonists⁵². This finding, and the absence of a magnesium effect in solubilised receptors, implies that coupling of receptors to adenylate cyclase is involved in the formation of the high-affinity agonist binding state. Confirmation of this comes from the finding that mutant lymphoma cells deficient in adenylate cyclase did not lose receptors on exposure to isoprenaline⁵³. In contrast, wild-type cells and mutants deficient in protein kinase, showed loss of β -receptors and adenylate cyclase responses after incubation

with isoprenaline, although the kinase mutants lacked the induction of phosphodiesterase seen in normal cells. Thus, activation of adenylate cyclase, but not changes in protein kinase and phosphodiesterase, appears to mediate the decrease in β -receptors and cyclic AMP responses of cells exposed to catecholamines. However, solubilised turkey erythrocyte membranes showed increased affinity for agonists, but not for antagonists⁵⁴, so that the formation of a high-affinity agonist binding complex may not depend only on coupling to adenylate cyclase.

Recently, comparison of agonist and antagonist binding assays has shown discrepancies in receptor numbers measured after β -adrenergic desensitisation of frog erythrocytes with isoprenaline *in vitro*⁵⁵. Whereas the loss of available sites measured with the labelled agonist ³H-HBI was correlated with loss of adenylate cyclase responses, binding of the labelled antagonist ³H-DHA was significantly less impaired. In a related study, desensitisation of frog cells by exposure to the radiolabelled agonist led to a loss of antagonist binding sites that exceeded the measured occupancy by residual agonist⁵⁶. These findings raise the possibility that prolonged high-affinity binding of agonist does not entirely account for the loss of β -adrenergic receptors measured by binding studies with labelled alprenolol. Whether this indicates that antagonist sites are regulated independently of agonist sites has yet to be determined. However, it is clear that occupancy of β -adrenergic receptors by agonist ligands is of higher affinity than anticipated from antagonist binding studies, and that *in vitro* desensitisation of adenylate cyclase is probably associated with sustained receptor occupancy.

A physiological role for regulation of β -adrenergic receptors was suggested by the observation that sympathetic control of *N*-acetyltransferase and melatonin synthesis in the rat pineal gland was modulated by marked changes in β -adrenergic receptor number and adenylate cyclase responsiveness during the diurnal light-dark cycle⁵⁷. Pineal glands of light-exposed rats contain more β -receptors and adenylate cyclase than glands from animals kept in the dark, and treatment with isoprenaline causes marked loss of both receptors and enzyme responsiveness to β -agonists. These changes seem to be involved in the varying sensitivity of the pineal gland during light-dark cycles, by modulating the capacity to form cyclic AMP in response to β -adrenergic stimulation. The desensitisation of pineal receptors by catecholamine, as in the frog erythrocyte⁴⁸, was not affected by cycloheximide, again suggesting that inactivation and reactivation, rather than receptor turnover, were responsible for the changing receptor concentrations. Modulation of β -adrenergic receptor binding of agonist ligands has also been noted in the myocardium, where guanyl nucleotides reduce binding affinity for agonist but not antagonist ligands⁵⁸. In this tissue, muscarinic cholinergic agents antagonised the GTP-induced reduction in β -receptor affinity for isoprenaline, and attenuated the combined effect of GTP and isoprenaline on myocardial adenylate cyclase. These findings suggest that cholinergic agonists can act through the muscarinic receptors to regulate both β -receptors and adenylate cyclase by modulating the effects of GTP. This action is consistent with the known ability of the cholinergic system to attenuate the cyclic AMP-mediated effects of sympathetic stimulation.

Regulation of gonadotropin receptors and gonadal steroidogenesis

In the Leydig cell of the testis and the luteal cell of the ovary, LH binds to plasma membrane receptors with high specificity and affinity ($K_d = 0.1$ nM). Hormone binding is followed by activation of adenylate cyclase and increased formation of cyclic AMP, which stimulates production of testosterone in Leydig cells and progesterone in luteal cells⁵⁹. Administration of ovine LH or hCG to rats is followed by prolonged loss of LH receptors in gonadal homogenates⁶⁰ and membrane preparations⁶¹, as well as in purified Leydig⁶² and luteal cells⁶³. The decrease of receptor number is both time- and dose-dependent⁶⁰⁻⁶⁴, and is

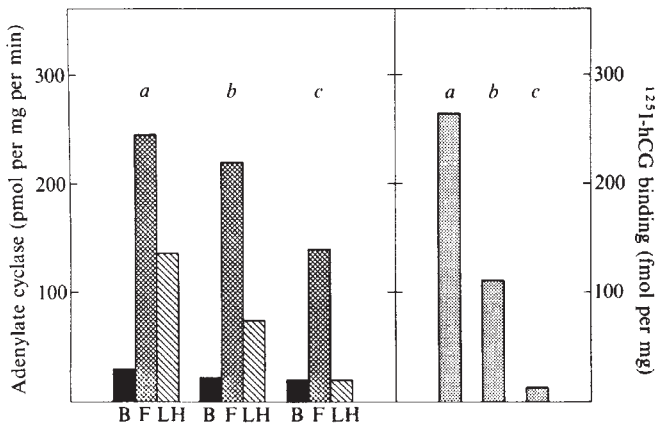


Fig. 2 Concomitant loss of LH receptors (right) and of adenylate cyclase responses to LH (left) in ovarian homogenates from rats treated 24 h earlier with 0.1 µg (b) and 20 µg (c) of hCG. The control levels of binding and adenylate cyclase (a) are shown at the left of each panel. For adenylate cyclase activity, basal (B), fluoride-stimulated (F) and gonadotropin-stimulated (LH) values are shown.

accompanied by prominent changes in the cyclic AMP and steroid responses of the desensitised cells^{63,65}. The expected effect on Leydig cell sensitivity to gonadotropins after receptor loss (an increase in the ED₅₀ for steroid production) is observed after subcutaneous treatment with hCG, as shown in Fig. 1. However, more marked receptor loss, particularly after intravenous treatment with hCG, is accompanied by a decreased maximum steroid response to subsequent hormone stimulation.

Of particular significance in studies of receptor regulation is the demonstration that the initial loss of LH binding capacity is due to occupancy of LH receptor sites by the desensitising dose of the hormone used to treat the animal^{61,65}. This has been shown by administration of ¹²⁵I-hCG, and directly by elution and radioimmunoassay of receptor-bound hCG after treatment with the unlabelled hormone. The initial occupancy stage, which lasts for up to 24 h, is followed by prolonged loss of LH receptors which is maximum after 1 to 2 d and lasts for up to 8 d in both testis⁶⁵ and ovary⁶⁶. After treatment with a low dose of hCG (200 ng), maximum receptor loss of about 60% is evident at 4 d. At this time, the decrease in binding cannot be due to occupancy, and represents depletion of LH receptors from the plasma membrane. The extent of true receptor loss is much greater than the initial occupancy by administered hormone^{61,65}, as also noted after growth hormone binding to cultured lymphocytes²⁹. These findings suggest that the initial occupancy induces an active or cooperative process in the cell membrane that augments the degree of receptor loss. In both testis and ovary, the period of net receptor loss is followed by return of the receptor number to normal over the next 6 to 10 d. The reappearance of gonadotropin receptors probably depends on synthesis of new receptor protein rather than recycling or reactivation of the lost receptor sites.

Receptor regulation in the testis and ovary has also been demonstrated following elevation of endogenous LH levels by administration of gonadotropin-releasing hormone (GnRH or LHRH) and its analogues. These studies also revealed marked and reversible loss of prolactin receptors, and regression of testis function⁶⁷⁻⁶⁹. Administration of GnRH to pregnant rats caused a fall in progesterone secretion and termination of pregnancy⁷⁰, probably due to functional luteolysis following loss of ovarian receptors for prolactin and LH, and desensitisation of the corpus luteum of pregnancy⁷¹. In addition, a direct effect of GnRH on the ovary may contribute to these regressive changes.

The disappearance of functional LH receptors, whether due to initial occupancy or subsequent receptor loss, is accompanied by decreased ability of LH to stimulate adenylate cyclase activity and cyclic AMP formation in the ovary⁶¹ and testis⁶⁵. At the time

of true receptor loss, the receptor number correlates with the degree of adenylate cyclase stimulation by LH in ovarian particles (Fig. 2). Subsequently, the recovery of receptors is accompanied by restoration of LH-stimulation of adenylate cyclase. Modulation of receptor number and adenylate cyclase stimulation has been shown with low doses of hCG, equivalent to the LH levels that occur during the normal reproductive cycle in the rat. Changes in adenylate cyclase responsiveness to LH after both induced and spontaneous ovulation^{72,73} suggest that receptor regulation and its consequences play a part in the regulation of ovarian function. Apart from their relevance to receptor regulation, these studies provide evidence that the binding sites measured with ¹²⁵I-hCG represent the functional LH receptors that are coupled to adenylate cyclase and the steroid activation mechanism.

The loss of adenylate cyclase responsiveness to gonadotropins during receptor occupancy shows that the gonadal enzyme, like that of the frog erythrocyte, becomes refractory after the initial hormone-receptor interaction. At this time, free LH receptors are still present but cannot undergo functional coupling to the catalytic unit of the enzyme. Transient loss of the fluoride effect on adenylate cyclase also occurs during desensitisation by hCG, with recovery in 24 to 36 h⁷⁴, corresponding closely with the occupancy phase of the LH receptors. Adrenaline can also stimulate adenylate cyclase in the ovarian luteal cell, and during hCG-induced desensitisation, when no loss of β-adrenergic receptors could be detected, there was decreased enzyme response to adrenaline⁷⁵. These findings suggest that hormone interaction with the LH receptor is followed by a transient refractory state in which the system cannot be stimulated by occupancy of free LH receptors or β-adrenergic receptors, or by fluoride. This refractory state is reversed within 24 h, as shown by the return of the fluoride and adrenaline responses. In contrast, the loss of LH receptors results in a prolonged loss of receptor-mediated LH activation of adenylate cyclase for several days.

Changes in Leydig and luteal cell LH receptors and adenylate cyclase activation have marked effects on the responses of the intact cell to gonadotropins^{62,63,76}. The production of cyclic AMP in desensitised cells is generally well correlated with changes in receptor number in both cell types, and steroid production is also reduced in proportion to receptor loss. In the ovary, treatment with 2 µg of hCG, enough to produce >90% reduction in the luteal LH receptors, caused almost complete loss of cyclic AMP and progesterone responses in isolated cells stimulated with gonadotropin *in vitro*. A lower dose of hCG (100 ng) caused a 50% fall in the receptor number and produced a similar decrease in both cell responses. In the Leydig cells of the testis, there was again a close correlation between decreased receptor number and loss of the steroid response to hormone stimulation. However, loss of steroid production was not fully accounted for by loss of LH receptors and the consequent failure of cyclic AMP generation, because addition of dibutyryl cyclic AMP or cholera toxin to over-ride the block in cyclic AMP synthesis failed to stimulate steroid production⁶². In the ovary, the loss of steroid response to hCG in partially desensitised cells was also seen with cholera toxin and dibutyryl cyclic AMP, and in fully desensitised cells these two stimuli had no effect on progesterone production⁶³.

The nature of the additional block beyond the level of cyclic AMP formation was characterised in the Leydig cells from hCG-treated rats. Depending on the desensitising dose of hCG, decreased conversion of steroid precursors to androgens was evident at specific points in the biosynthetic pathway⁷⁷. Although high doses of hCG caused a decrease in the formation of pregnenolone from cholesterol, lower doses of hCG did not impair the side-chain cleavage step while still causing a marked fall in androgen production. Detailed analysis of the testosterone biosynthetic pathway indicated that the 17, 20 desmolase step becomes rate-limiting in the desensitised state, with accumulation of 17-hydroxylated precursors. Also, reductions in 3β-hydroxysteroid dehydrogenase and 17-hydroxylase activi-

ties were suggested by the increased levels of pregnenolone and progesterone formed in desensitised cells. The mechanism by which gonadotropin treatment causes these reductions in enzyme activity has not been elucidated, but may depend on local inhibitory feedback by oestrogen produced during the initial stimulation by the desensitising dose of hCG. It is also likely that maintenance of the steroid biosynthetic pathway requires the 'trophic' influence of LH, and that receptor loss impairs this long-term action of gonadotropins as well as their acute effect on steroid biosynthesis and secretion.

Regulation of angiotensin II receptors and adrenal function

The renin-angiotensin system plays a major part in the control of aldosterone secretion during changes in electrolyte intake and sodium balance. This action is exerted through the binding of angiotensin II to specific receptors in the adrenal zona glomerulosa cells, with stimulation of aldosterone production via a non-cyclic AMP-dependent mechanism. In addition to this acute action on steroid secretion, the renin-angiotensin system exerts an important trophic action on the glomerulosa zone, as seen in prolonged sodium deficiency. The adrenal angiotensin II receptors are predominantly in the glomerulosa layer, and exhibit high-affinity binding of agonist and antagonist derivatives of angiotensin II, with association constants in proportion to their biological activities.

Angiotensin II receptors are regulated by the prevailing concentration of the peptide ligand³⁵, and also by changes in electrolyte intake⁷⁸. The latter action is at least partly exerted through changes in blood angiotensin II concentration. Decreased sodium intake and increased potassium intake, which both markedly elevate aldosterone secretion, also cause increased angiotensin II receptor content in the glomerulosa cell⁷⁸. Conversely, high sodium or low potassium intake, which both reduce aldosterone secretion, cause a decrease in adrenal receptors for angiotensin II. The sodium-induced changes are largely attributable to the altered renin secretion and angiotensin II concentrations that accompany variations in sodium intake. Thus, blood angiotensin II levels are increased in sodium deficiency and decreased in sodium loading, and both adrenal angiotensin II receptors and aldosterone secretion are commensurately altered by changing sodium intake. Changing potassium intake has the opposite action to sodium on angiotensin II levels, but the same effect on glomerulosa cell receptors and aldosterone production, by an independent direct effect of potassium on the zona glomerulosa.

During sodium deficiency, the adrenal becomes more sensitive to angiotensin II, whereas vascular smooth muscle becomes less sensitive and pressor responses to the octapeptide are blunted. These changes partly depend on altered receptor properties, as even short periods (36 h) of sodium deficiency caused increased binding of angiotensin II by the rat adrenal *in vivo* and *in vitro*, whereas sodium loading had the opposite effect⁷⁹. Enhancement of binding by sodium restriction was due to a rapid increase in receptor affinity, followed within a few days by increased receptor content. Conversely, sodium loading resulted in a fall in the number of angiotensin II binding sites per adrenal glomerulosa cell. In each case, the aldosterone responses of isolated glomerulosa cells were commensurate with the changes in angiotensin receptors. Thus, cells exhibited increased sensitivity to angiotensin II when receptor affinity was raised, increased maximum aldosterone secretion when receptor content became elevated, and decreased responses when receptor content was diminished. These changes in receptors and steroidogenic activity of the glomerulosa cells contribute to the regulation of sensitivity and maximum responsiveness of the adrenal to angiotensin II during altered sodium balance. The presence of a converse effect in smooth muscle receptors during low sodium intake was consistent with the reduced sensitivity of vascular smooth muscle to angiotensin II during sodium deficiency. The contrasting effects of low sodium intake on adrenal and uterine smooth muscle receptors are shown in Fig.

3, the reciprocal changes in receptors being parallel to the altered sensitivity of each tissue to angiotensin II.

The trophic actions of angiotensin II in the glomerulosa cell include effects on angiotensin II receptors and enzymes in the steroid biosynthetic pathway, as well as on glomerulosa cell differentiation. Infusion of angiotensin II enhances adrenal sensitivity to the peptide in man and rat, and increases the angiotensin II receptor content of the rat adrenal, with an accompanying rise in aldosterone secretory capacity³⁵. Although injection of large doses of angiotensin II can decrease the adrenal and uterine receptors in nephrectomised rats^{80,81}, treatment of intact rats with smaller amounts of angiotensin II has the converse effect, increasing angiotensin II receptors and aldosterone responses in the adrenal glomerulosa cell³⁵.

The ability of angiotensin II to cause positive regulation of its own receptor sites in the adrenal suggests that most or all of the effects of sodium deficiency on aldosterone secretion could be mediated by changes in angiotensin II secretion and adrenal sensitivity to angiotensin. This controversial concept has recently been supported by the results of direct assay of adrenal receptors and glomerulosa cell responses⁷⁹. Further evidence has been provided by the use of an inhibitor of converting enzyme to prevent angiotensin II formation during sodium restriction, which would normally cause increased adrenal receptors and aldosterone production⁸². Blockade of angiotensin II generation during sodium restriction completely abolished the increased receptor binding and aldosterone responses that occur in sodium deficiency, indicating the importance of angiotensin II in mediating the adrenal response to altered sodium balance. This finding emphasises the central role of the renin-angiotensin system in the regulation of aldosterone secretion during short-term physiological changes in sodium intake.

Fate of the hormone-receptor complex

Most studies of peptide hormone action have concentrated on the acute responses that follow the binding of hormone to its cell-surface receptor. Much less is known about the equally important long-term actions on cell growth and differentiation, or about the termination of hormonal stimuli. These events may be related to the fate of the hormone-receptor complex. As noted above, there is evidence that the initial interaction is followed by formation of a higher-affinity binding state that causes the complex to remain associated for prolonged periods. That, together with increasing evidence for the presence of hormones and binding sites within target cells, has led to the

Table 1 Formation and degradation of the hormone-receptor (H-R) complex

Formation and actions of the H-R complex

- A H-R becomes coupled to ACy or other effectors to form H-R-ACy
- B H-R-ACy initiates acute response to hormone
- C H-R-ACy complex becomes refractory (desensitisation)
- D H-R becomes uncoupled from ACy

Fate of H-R complex

- A H released, R re-utilised with turnover independent of occupancy (traditional)
- or
- B H-R released from cell surface (shedding)
- or
- C H-R internalised
 - 1. Loss of occupied surface receptors (down-regulation)
 - 2. Concomitant endocytosis of adjacent unoccupied receptors
 - 3. Lysosomal degradation of H, possibly of H-R
 - 4. Mediates intracellular trophic action
- D H metabolised or inactivated, R remains in inactive (non-binding) form

The most clearly demonstrated mechanism of receptor regulation is by internalisation of the H-R complex. The other pathways could operate in specific tissues and their relative contributions probably vary with the functional state of the cell. ACy, adenylate cyclase.

view that internalisation is a frequent consequence of hormone-receptor interaction. Interest in this process was stimulated by demonstration of the uptake and turnover of cholinergic receptors⁸³ and low-density lipoprotein-receptor complexes⁸⁴. Subsequently, increasing emphasis has been placed on the importance of internalisation in the processing of hormone-receptor complexes in endocrine target tissues. The mechanism of the internalisation process for peptide hormones probably has many features in common with the processes of surface modulation in lymphocytes² and the coated pit system responsible for uptake of macromolecules such as LDL⁸⁵. The basis of the increase in target cell receptors induced by peptides such as angiotensin II and prolactin requires further study but is likely to be related initially to changes in membrane conformation and later to increased receptor synthesis.

Several studies have implicated cytoplasmic contractile elements in receptor mobility and regulation. In lymphoid cells, ligand-receptor interaction is followed by patch formation, aggregation into a few larger patches or a single cap, and finally by internalisation or shedding of the cross-linked receptors. Examination of surface receptor capping by fluorescence staining of the receptor-bound ligand and intracellular actin or myosin showed that patching of surface antigens in splenic T lymphocytes (and lectin receptors in HeLa cells) was accompanied by collection of actin or myosin beneath the aggregated receptors⁸⁶. In the presence of sodium azide, the ligands induced a patchy redistribution of receptors and a concomitant rearrangement of membrane-associated actin on the cytoplasmic surface of the cell membrane. In the absence of azide, the patched receptors became capped, with accumulation of the underlying actin and myosin beneath the cap. These observations, and previous findings on Ig receptor capping in B lymphocytes⁸⁷, led to the proposal that a general mechanism of capping depends on the collection together of patches by actin and myosin, analogous to the sliding filament mechanism of muscle contraction⁸⁶. This mechanism also provides a plausible link between capping and subsequent endocytosis, by the concerted action of actin and myosin to initiate invagination of the capped region of the cell membrane. Cellular contractile elements have been also implicated during capping of lectin receptors in cultured ovarian granulosa cells⁸⁸, in which microfilaments were active in the redistribution of surface receptors during capping, and microtubules had an orientating effect on the direction of flow of receptor complexes.

Although receptor patching and capping commonly occur in cells exposed to multivalent ligands such as immunoglobulins and lectins, the progression from patching to capping may not apply when receptors are occupied by univalent ligands such as hormones and transmitters. However, the processes leading to aggregation and internalisation of multivalent ligands may be relevant to the turnover and fate of peptide hormone receptors, particularly during receptor regulation in response to increased concentration of hormonal ligands. In recent studies, described below, redistribution of receptors for hormones and other univalent ligands has been found to precede internalisation of receptor aggregates and subsequent degradation of the complexes.

The association of hormonal internalisation with ligand-induced receptor loss was clearly documented for epidermal growth factor (EGF) in human fibroblasts⁸⁹. The cell-bound mitogen was rapidly degraded in a manner consistent with internalisation and lysosomal breakdown, and surface receptors for EGF simultaneously disappeared from the cells. Loss of EGF receptors after peptide binding was also demonstrated by affinity labelling, which showed that rapid proteolytic processing of receptor-hormone complexes accompanied the decrease in membrane receptor sites⁹⁰. Also, both receptor processing and DNA synthesis were stimulated half-maximally when 10% of the receptors were occupied by the mitogen. However, in cultured ovarian cells the luteinisation of granulosa cells was associated with increased EGF receptors and retention of the ability to internalise bound EGF, but with loss of responsiveness

to the mitogenic action of EGF⁹¹. At least in these cells, there was no correlation between mitogenic activity and the capacity for EGF binding and internalisation.

Direct visualisation of the internalisation of fluorescent analogues of EGF and insulin has been achieved by image intensification during fluorescence microscopy⁹². The hormone-receptor complexes were initially distributed uniformly on the cell membrane, then rapidly became aggregated into patches that were internalised to form endocytic vesicles within the cytoplasm. These events could explain both the negative cooperativity of insulin receptors and the down-regulation of receptors and hormone degradation by target cells. Binding and internalisation of EGF has also been observed by conventional fluorescence microscopy in epithelioid carcinoma cells with 25 to 30 times more EGF receptors than in fibroblasts⁹³. The bound EGF was again rapidly internalised as fluorescent endocytic vesicles, which later formed a prominent perinuclear ring. Although patch formation did not occur in the epithelioid cells, the occurrence of micro-clusters of receptors was demonstrated by electron microscopy after binding of ferritin-EGF to the cell surface (S. Cohen, personal communication).

These observations on internalisation of EGF are probably applicable to other peptide ligands, although the probability of differences between *in vitro* and *in vivo* regulation should be noted, as should the degree of variability in the rate and extent of receptor processing in various cell types. This is illustrated by the fate of receptor-bound insulin after uptake by adipocytes, lymphocytes and hepatocytes. The specific binding of ¹²⁵I-insulin to adipocytes became less reversible with time, as if compartmentalised and less accessible to dissociating agents⁹⁴. Also, the bound tracer was rapidly converted to iodotyrosyl fragments, suggesting that internalisation and processing is responsible for the degradation of much of the insulin bound to receptor sites. In contrast to the cultured lymphocyte, where translocation was minor, insulin bound to hepatocytes was progressively internalised and underwent lysosomal degradation⁹⁵. This process must have occurred by endocytosis of the hormone-receptor complex, and would explain ligand-induced receptor loss. However, down-regulation of insulin receptors is also prominent in cultured lymphocytes, where the intracellular translocation of hormone is small in relation to the final degree of receptor loss. Given the time required for insulin-induced receptor loss in cultured lymphocytes, the rate of internalisation could be adequate to account for the receptor loss observed after several hours in the cultured cells.

Further evidence for hormone internalisation was provided by studies on the uptake and metabolism of gonadotropins in cells of the testis and ovary. The presence of gonadotropin binding sites in the cytoplasm of ovarian cells has been demon-

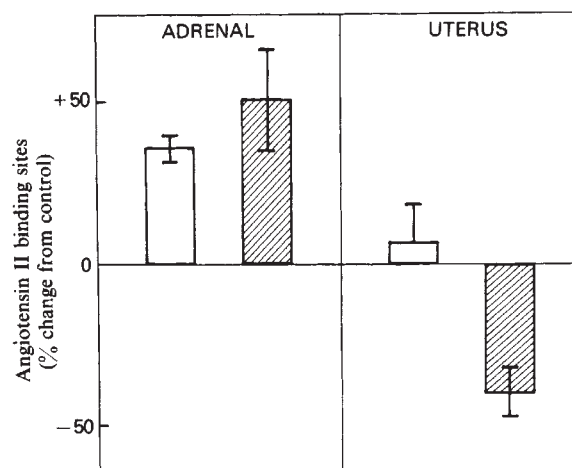


Fig. 3 The reciprocal effects of low sodium intake on angiotensin II receptors of adrenal glomerulosa cells and uterine smooth muscle. Open column, 36-h low sodium; cross-hatched column, 7-d low sodium.

strated by immunocytochemistry and autoradiography. After *in vivo* treatment with hCG, localisation of the hormone was demonstrated in membrane, cytoplasmic and perinuclear sites, much of the hormone being within the cytoplasm³. More direct evidence has come from studies of the ovine corpus luteum, where significant amounts of ¹²⁵I-hCG were taken up within endocytic vesicles and incorporated into dense bodies, presumably lysosomes⁹⁶. Lysosomal degradation of gonadotropins occurs in Leydig tumour cells, in which the binding and metabolism of ¹²⁵I-hCG was not affected by agents which alter microfilaments and microtubules⁹⁷. In these cells, steroidogenesis is not dependent on hormone degradation, consistent with the rapidity with which binding to receptors is followed by the acute metabolic effects of the hormone. The loss of gonadotropin receptors induced by hCG in the ovary is accompanied by progressive internalisation of receptor-bound ¹²⁵I-hCG, and at least a proportion of the translocated radioactivity can be identified as the hormone-receptor complex after solubilisation and density gradient centrifugation⁹⁸. Such findings have indicated that internalisation is a common sequel to hormone-receptor interaction, and is likely to be responsible for the down-regulation of surface receptors that frequently follows exposure to elevated concentrations of the homologous hormone.

Conclusions

The effects of receptor regulation on target cell function are both marked and widespread in peptide-modulated endocrine tissues. The initial phase of receptor occupancy and target cell activation is frequently followed by a period of diminished responsiveness that accompanies the presence of residual hormone on receptor sites. This corresponds to the short-term phenomenon described variously as desensitisation, tachyphylaxis or refractoriness, and is sometimes caused by temporary inactivation of adenylate cyclase. In the case of non-cyclic AMP-mediated hormones, other membrane effector systems are presumably inactivated during receptor occupancy. A general scheme of the sequence of events following hormone-

receptor interaction is shown in Table 1, which presents the known and hypothetical events that follow the initial event of activation by binding of the homologous hormone to the specific membrane receptors.

In certain tissues, such as the amphibian erythrocyte, refractoriness may depend largely on such transient inactivation of receptors and adenylate cyclase, with subsequent reactivation of these membrane systems when the agonist-receptor complex dissociates. In other cells, depending on the affinity with which the ligand is bound and the rate and extent of receptor and membrane turnover, occupancy is followed by a true loss of receptor sites due to processing and internalisation of the hormone-receptor complex. The degree to which different ligands are able to decrease or increase their specific receptors must also depend on the relative rates of receptor processing and resynthesis in the respective target cells. Thus, minor loss of receptors may not be evident if the rate of receptor synthesis or activation is markedly increased by the initial hormonal stimulus, a mechanism that could account for the increased receptor number in certain cells exposed to high hormone levels. In the more general case, where marked loss of receptor sites follows hormone binding, it is likely that peptide hormone receptors are not necessarily vacated and re-utilised, but may be occupied only once and then degraded or processed.

The fate of processed hormone-receptor complexes, and perhaps some of the adjacent free receptors, probably depends on internalisation and lysosomal degradation. The initial event involves incorporation of both hormone and receptors into endocytic vesicles probably through a coated pit mechanism, with subsequent rapid metabolism of the hormone. Whether recycling of membrane components occurs during receptor regeneration is unclear, but this seems unlikely given the prolonged time needed for recovery of receptors in most tissues. The biological role of hormone-receptor internalisation and degradation remains uncertain, other than as a mechanism for terminating hormone action initiated by tightly bound ligands. These processes may also protect target cells from prolonged or excessive stimulation, and could exert long-term effects on target cell growth and differentiation.

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articles

Accretion temperature of the Tieschitz, H3, chondritic meteorite

R. Hutchison & A. W. R. Bevan

Department of Mineralogy, British Museum (Natural History), London SW7, UK

S. O. Agrell

Department of Mineralogy and Petrology, Downing Place, Cambridge, UK

J. R. Ashworth

Department of Geological Sciences, The University of Aston, Birmingham, UK

The Tieschitz, H3, chondritic meteorite apparently formed as part of a parent-body by the accretion of both molten and solid materials with a mean temperature of 800 ± 100 °C, followed by slow cooling to lower temperatures. Our data argue against the currently accepted model of equilibration with a cooling gas of solar composition.

KNOWLEDGE of the conditions of planetary formation in the Solar System is largely obtained from meteorite studies. Of particular interest are the unequilibrated chondrites, rare meteorites in which disequilibrium mineral assemblages have survived because they were unaffected by the period of thermal metamorphism which produced crystallisation in most chondrites. Tieschitz fell in Czechoslovakia in 1878. It belongs to the high iron, H, group of chondrites, has prominent chondrules many of which contain glass, and its olivines and pyroxenes have wide ranges of composition. Tieschitz is, therefore, an unequilibrated chondrite *par excellence*^{1,2}. All chondrites contain small amounts of the volatile metals Ti, Bi and In, whose presence is explained either by the accretion of various proportions of high temperature and low temperature fractions, followed by closed system metamorphism^{3–5}, or else by total accretion at low temperature followed by open system metamorphism^{6,7}. In either case, models are based on the condensation of volatiles from a gas of solar composition. Recently, Ikramuddin *et al.*⁸ heated aliquots of powdered Tieschitz for a week at different temperatures and under a low pressure of hydrogen. Ten elements were determined in each aliquot and elemental loss from unheated material was calculated for each temperature. It was concluded that, in the experimental conditions, open system metamorphism of Tieschitz starting material could not account for trace elemental abundances in more metamorphosed chondrites.

We have carried out some 600 analyses of Tieschitz silicate minerals, glasses or fine-grained intergrowths, sulphide and metal. One of us (J.R.A.) has used high-voltage electron microscopy to study textures and identify minerals in the fine-grained matrix. Mobility during accretion of nepheline-normative liquids allows us to use data from experimental petrology to constrain accretion temperatures. Finally, we discuss the implication that, because of the presence of troilite, the Tieschitz assemblage was not in equilibrium with a gas of solar composition^{9,10}.

Results

The meteorite comprises millimetre fragments and spherical chondrules of silicate, all of which have opaque rims, together with smaller, near spherical bodies of metal or metal plus troilite. Outside the opaque rims, between fragments and chondrules, there may be a channel of variable width filled by transparent material; this has been termed by Christophe-Michel-Levy¹¹ "white matrix", in contrast to the "black matrix" of the opaque rims. A few of the fragments clearly represent pieces broken from solid, chemically fractionated, pre-existing rock. For example, one fragment is composed of a granular intergrowth of pigeonite, olivine (Fo₈₆), and crystalline plagioclase (An_{78–68}); this rock is a norite. However, other angular fragments have minerals of variable composition set in devitrifying glass and were apparently broken from solid chondrules or pre-existing rock.

Most chondrules exhibit quench textures. Olivine and/or pyroxene crystals are associated either with clear glass or with fine-grained turbid material which is interpreted as devitrified glass. Some chondrules contain blobs of metal or sulphide or both. Most olivines and pyroxenes are zoned. In all cases zoning is normal, olivines tending towards iron enrichment in rims and pyroxenes tending towards iron and/or calcium enrichment. In

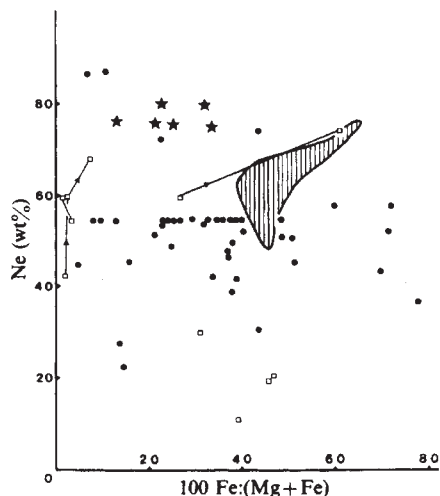


Fig. 1 'Nepheline' (wt %) versus 100 Fe:(Fe+Mg) (atomic), in Tieschitz glasses and fine-grained matrices. 'Nepheline' is the figure obtained from casting the bulk analysis in the SiO_2 , nepheline, kalsilite residual system (see Fig. 2). Shaded area represents the white matrix. ●, Chondrule glasses and matrices. ★, Lilac glass from dumb-bell-shaped chondrule. □, Inclusions in olivine; tie lines link those from the same host crystal. A row of solid circles has normative feldspar close to albite, which projects at 54% Ne.

composition, olivine cores in 30 chondrules or rock fragments range from pure forsterite to Fo_{60} . Seven isolated olivines from the matrix between chondrules are either Fo_{80} or about Fo_{60} ; in addition one matrix olivine is Fo_{90} .

Glasses or turbid mesostasis in 30 chondrules or chondrule fragments were analysed. Compositions vary from chondrule to chondrule, and, to some extent, within chondrules. The atomic 100 Fe:(Fe+Mg) ratio ranges from 4 to 80 (Fig. 1) and the lime content (wt) from about 1% to over 14%. Analyses were performed using an energy dispersive system on an electron microprobe. Usually, the energy spectrum was integrated over 80 s on a single point, but in some cases a defocused beam was used and in others the spectrum was integrated for four 20-s counts each on a different spot. There was no evidence of loss of alkalis, and glasses apparently did not decompose under electron bombardment; totals were always between 96 and 102%. Figure 2 shows the analyses cast in the silica-nepheline ($\text{NaAlSi}_3\text{O}_8$)-kalsilite (KAlSi_3O_8) ternary diagram (wt), after assigning SiO_2 to the various normative mafic and calcium-bearing minerals. All interstitial materials within chondrules plot close to, or on, the SiO_2 -nepheline join, because of their lack of K_2O . However, in some chondrules, phenocrysts of olivine contain inclusions, some of which have over 1.5% K_2O . In one pair of inclusions and one triplet, each from a single olivine, a decrease in K_2O is accompanied by an increase in the iron-to-magnesium ratio. This negative correlation of the K_2O and the iron-to-magnesium ratio is contrary to the normal consequences of igneous fractionation. Hence we believe that, in some chondrules, loss of potassium by volatilisation took place. Early, potassium-rich liquids were preserved by their complete enclosure in olivine. On the ternary plot, about half of the chondrule matrices project at the albite composition; in this respect many Tieschitz chondrules resemble those of Bjurböle¹².

A small proportion of the chondrules have non-spherical shapes which were determined, after accretion, by the impingement of adjacent objects on them. At least some of these mis-shapen chondrules were undoubtedly still partially molten during aggregation. One encountered in the present study is roughly dumb-bell-shaped (Fig. 3) and has skeletal crystals of olivine set in turbid, lilac-coloured glass. The olivines are close to pure forsterite, but the glass shows some iron enrichment; this latter is highly nepheline-normative and contains up to about

0.5 wt % K_2O ; analyses gave totals from 98 to 102%. Texturally it seems evident that this material was plastic when incorporated into the meteorite parent. Subsequent compaction by other, more solid objects caused it to adjust by flow to fit the irregular space available. A second mis-shapen chondrule (Fig. 4) is micro-crystalline. It too, was clearly plastic or molten when accreted. Subsequent deformation caused the dark rim to rupture as part of the contents squeezed out. The micro-crystalline material is potassium-poor, with variable under-saturation in silica. The 100 Fe:(Fe+Mg) atomic ratio also varies from 45 to 70. Some decomposition occurred under the electron beam and totals were rather low, with a maximum of 97.5; we suspect that the fine-grained material may be slightly hydrated, as the surface is highly polished.

White matrix occupies some narrow, interstitial areas outside the dark rims of chondrules and clasts¹¹. In thin section, between crossed polars, it consists of a fine-grained intergrowth of crystals of low birefringence; electron diffraction indicates that nepheline is common and some plagioclase is present. Only broken grains were found in the electron microscope study, but some large ($>10\ \mu\text{m}$) fragile nepheline fragments were probably generated close to their present location. A polished surface in reflected light confirms that white matrix is composed of angular to rounded fragments, the largest observed being close to $20\ \mu\text{m}$ across. The bulk compositions of compact white matrix (Fig. 2) indicate the same nepheline plus plagioclase mineralogy. Textural evidence (Fig. 5) shows that cohesive masses of this white matrix were mobile during the accretion of the chondrite. Analysis totals for white matrix are low (80–97%; compare ref. 11), because it does not become highly polished (due to its fractured nature?), and decomposes in the electron beam. Nevertheless, compared with chondrules, our data (analyses of 25 locations) establish a striking consistency of composition, throughout a 2.5-cm^2 thin section (Figs 1, 2). White matrix usually has 8–10% Na_2O , about 2% CaO and 100 Fe:(Fe+Mg) of 40–60.

Discussion

It seems that some constituents of Tieschitz were molten or plastic after aggregation. Experimental petrology may be used to constrain the conditions in which flow could have taken place. We can then outline some of the consequences imposed by these conditions for the cooling history of the meteorite.

There are two temperature minima on the silica-nepheline join, separated by the albite ($\text{NaAlSi}_3\text{O}_8$) composition (Fig. 2). On cooling, liquids with compositions between albite and silica crystallise one or other phase, depending on initial liquid composition. This drives the composition of the residual liquid towards the minimum at which both phases crystallise together at $1,062^\circ\text{C}$ (for the dry system) until completely solidified¹³. For

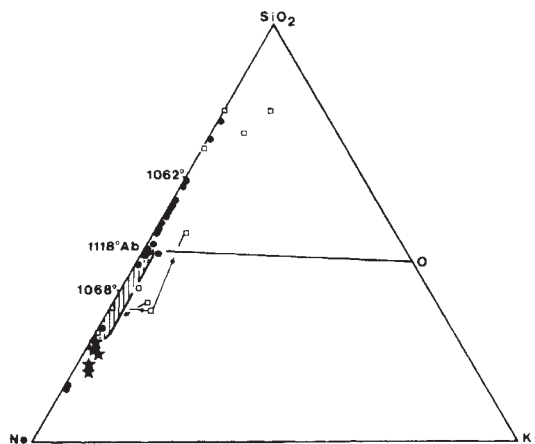


Fig. 2 Residual system SiO_2 -nepheline ($\text{NaAlSi}_3\text{O}_8$)-kalsilite (KAlSi_3O_8) (wt). Symbols as in Fig. 1. The melting point of albite, and minima on the SiO_2 -nepheline join are in $^\circ\text{C}$.

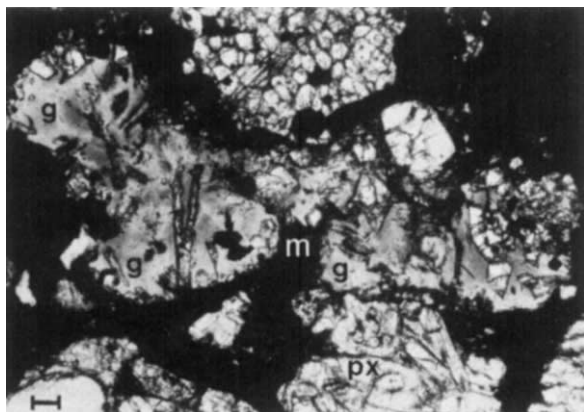


Fig. 3 Dumb-bell-shaped chondrule. m, Metal grain in dark matrix; g, lilac-coloured glass, partly turbid; px, porphyritic pyroxene chondrule. Although some brittle fracturing occurred above and to the right of 'm', the two long, fragile olivine crystals between 'g' and 'm' (lower left of centre) are undeformed and grew *in situ* from the melt after the shape of the chondrule had been determined. Scale bar, 0.05 mm.

liquids with composition between albite and nepheline, crystallisation of one of these phases drives the liquid towards a minimum at 1,068 °C. Because white matrix compositions straddle this second minimum, they probably represent solidified, fractionated liquids. Between the two minima is a thermal barrier which cannot be crossed by a crystallising liquid at low pressure. We interpret the white matrix as being representative of different liquids which tended towards the 1,068 °C minimum by crystal-liquid fractionation. On the other hand, many chondrule liquids are oversaturated with silica and so tended to fractionate towards the 1,062 °C minimum. But these liquids are more viscous than the undersaturated ones and hence were not mobile during accretion.

We envisage that white matrix liquids were available in small quantity distributed among the aggregating mass of chondrules, clasts, metal, sulphide and mineral fragments. These liquids show a genetic relationship with nepheline-normative chondrules. Initially, white matrix must have been sufficiently mobile for it to have flowed for distances of a few mm along inter-chondrule channels up to 50 µm wide. The question then arises in what conditions could the white matrix have been mobile? One measured composition (SiO₂ 56.2% (wt); TiO₂ 0.50; Al₂O₃ 18.3; FeO 4.75; MgO 3.32; CaO 3.86; Na₂O 8.9; K₂O 0.40; sum 96.23), with 1 wt% H₂O, has a calculated minimum viscosity of 10⁴ P at 900 °C (H. Pinkerton and L. Wilson, personal communication, and refs 14, 15). Water is present in Tieschitz¹; 1% water in white matrix lowers the viscosity by a factor of 2–5, and slightly lowers the liquidus temperature. The calculated viscosity is comparable to that of rhyolite liquids during eruption; their viscosities are so high that it is surprising that they can be erupted at all. A liquid of 10⁴ P requires a pressure gradient of about 50 kbar cm⁻¹ to cause it to flow for 5 mm along a channel 25–50 µm wide. The pressure required is greatly in excess of the measured compressive strengths of meteorites¹⁶, hence injection of white matrix as a liquid at 900 °C or less, is impossible. The calculated yield strength of liquid white matrix at 900 °C is 10⁸ c.g.s. units¹⁵. Emplacement as a solidifying liquid, by comminution, requires a pressure gradient of 20 kbar cm⁻¹ which is still unacceptably high. Crystallisation would exacerbate the situation. The Tieschitz meteorite is, in fact, noted for the absence of significant deformation effects¹¹. We must therefore assume that white matrix was emplaced in a fluid state which requires a temperature above 900 °C. Now, a 100-µm thick film of silicate liquid, in a vacuum, would solidify from 1,100 °C in microseconds. So, if Tieschitz accreted mainly as chondrules, clasts and metal at 170 °C (~455 K, for the two-component model championed by Anders and co-workers¹⁷), interspersed with a few per cent of

molten chondrules and white matrix liquid, the melts would have solidified immediately.

Our only solution to this problem seems to be that hot, liquid, white matrix and plastic chondrules were accreted with chondrules, clasts, metal and sulphide at a cooler, though still high, ambient temperature. We now try to constrain this temperature. Chondrules show the effects of rapid quenching and we observe gross compositional heterogeneities between olivine and pyroxene crystals. The main, low-calcium pyroxene is the twinned monoclinic variety. There is also pigeonite exsolved on a very fine scale (~10 nm) in crystals with a higher lime content. All these features indicate rapid cooling to around 800 °C, or less. In addition, low-calcium pyroxenes with a variety of iron contents have remained unattacked by engulfing white matrix which, being nepheline-normative, must have been strongly out of equilibrium with this pyroxene. Either the chondrules and debris came together at about 800 °C, with residual alkaline liquids at higher temperature and still crystallising matrix olivine (Fo_{60–80}), or accretion took place with all the components at temperatures above 800 °C, during rapid cooling. In either case we propose that the effective accretion temperature was 800 °C, this being the highest temperature that can plausibly be invoked for the end of rapid cooling of the mafic silicates.

An upper limit to the accretion temperature is fixed at 988 °C by the minimum in the Fe–FeS system¹⁸. The addition of nickel lowers the minimum, but precise data are not available. If the ambient temperature during accretion of Tieschitz had exceeded 900 °C, then metal plus sulphide should have been mobilised¹⁹, and squeezed between the chondrules. As this is not observed, we can set the maximum temperature during accretion at 900 °C. Because the Fe–FeS eutectic temperature is well below the solidus on the nepheline–albite join, we can eliminate metamorphic reheating as a cause of white matrix mobility. Had the Tieschitz assemblage aggregated cold, subsequent heating would have caused the flow of metal plus sulphide liquids when the eutectic was reached at above 900 °C. A further increase of over 100 °C would have been required to produce melting in the white matrix. We have observed no textural evidence of metamorphism except for compaction in some chondritic clasts. In them the same unequilibrated silicates occur as in the meteorite as a whole. A lower limit to the accretion temperature may be established as follows: metal in Tieschitz occurs in a number of textural associations. Nickel distribution in the metal grains was measured using a con-

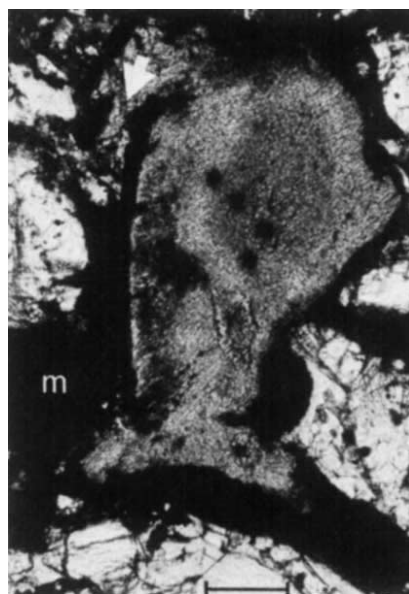


Fig. 4 Mis-shaped chondrule. m, Metal. Compression from left and right caused the dark matrix surrounding the chondrule to rupture, forcing the contents out (see arrow). The four dark spots are 'probe' burns. Scale bar, 0.05 mm.

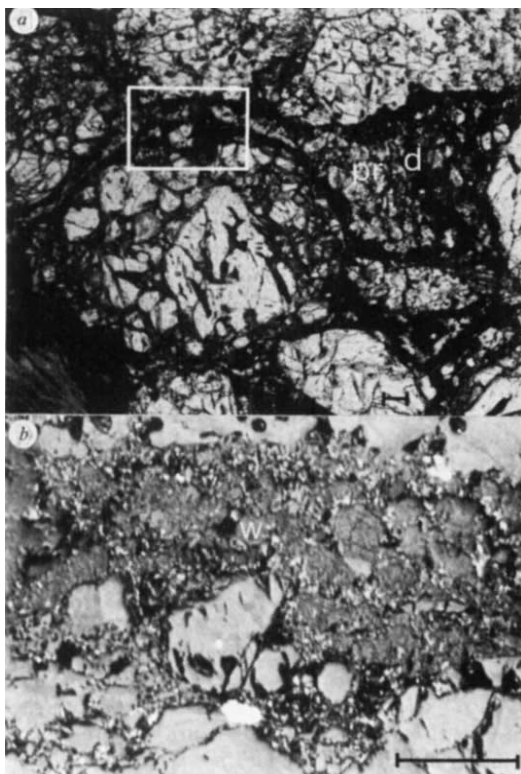


Fig. 5 White matrix: *a*, transmitted light; *b*, reflected light. The field of *b* is the rectangular outline in *a*. In the top left of *a* white matrix fills channels between three chondrules outlined by dark matrix. To the right, one channel with white matrix runs into a pyroxene-rich area *pr*, which is delimited on the right by dark matrix *d*. *b* Illustrates the block-like nature of white matrix, one piece of which is marked (*w*). Metal is brilliant white. Vermicular, light lines are cracks outlined by adhering carbon coat which was cleaned from most of the surface. Scale bars, 0.05 mm.

ventional, wavelength-dispersive microprobe technique. Both kamacite and taenite grains show a relationship between size and central nickel content, indicating that all metal cooled through about 500 °C *in situ*, thus confirming the conclusion of Wood²⁰. The cooling cycle must have begun near 700 °C, the temperature at which bulk Tieschitz metal (11.8% Ni, ref. 21) would enter the ($\alpha + \gamma$) field, if the effect of undercooling is neglected. Thus the lower limit to accretion temperature is fixed at 700 °C. We can therefore conclude that Tieschitz effectively accreted at (800 ± 100) °C.

Christophe-Michel-Levy argued that white matrix represents part of the contents of chondrules which had been leached out and redeposited by a fluid phase¹¹. However, it seems impossible to mobilise preferentially a silica undersaturated component, white matrix, without attacking low-calcium pyroxene. (For example, in the terrestrial, aqueous environment low-calcium pyroxene tends to be very unstable.) Moreover, the presence of water could not have been responsible for the deformation of the chondrule in Fig. 4, because the effect of water in reducing the viscosity of the chondrule liquid would have been much less than the effect of falling temperature in increasing it.

Accretion of an unequilibrated ordinary chondrite at 800 °C has several implications. This temperature is higher than that of the reaction of H₂S gas with metal to form troilite plus hydrogen from a gas of solar composition^{9,10}. We must then assume that any gas in equilibrium with the accreting components of Tieschitz was depleted in hydrogen relative to a gas of solar composition¹⁰. Obviously, this can be overcome by *ad hoc* assumptions such as rapid formation of chondrules and metal plus sulphide during, for example, an impact event—but this must be a non-equilibrium reaction. In contrast, the story in the Tieschitz metal supports equilibration and slow cooling over

temperatures around 500 °C, which is still above the temperature at which troilite can form by reaction from a gas of solar composition. Following Herndon and Suess¹⁰, our findings indicate that the Tieschitz assemblage was probably associated with a gas depleted in hydrogen by a factor of 100 or more relative to solar composition.

These implications may be taken further. In the two-component model, or variations of it, the temperature of accretion of chondrites has been defined by the estimated temperature at which a low temperature, volatile-rich component ceased to equilibrate with a cooling gas of solar composition. Larimer's estimate for Tieschitz is ≤445 K (170 °C)¹⁷, it depends, among other things, on gas composition. A temperature of 170 °C may be reconciled with our estimate of 800 °C for the accretion temperature if it is assumed that the highly porous Tieschitz^{11,22} continued to equilibrate with gas during slow cooling. During this stage of the meteorite's history the partition of Ni between kamacite and taenite occurred. In addition the meteorite received its complement of the volatile metals Ti, Bi, In and Pb by condensation from the coexisting gas. Such a mechanism has been suggested for the L3 chondrite, Khohar, on the basis of Bi distribution between metal grains²³. Where did the volatile metals go? They probably condensed into any suitable substrate, such as dark matrix around chondrules and clasts. Dark rims (=matrix) to chondrules and clasts were, in some instances at least, picked up when chondrules were liquid. We cannot, therefore, assume that the volatiles are present in the dark rim material, or that a low temperature carrier of volatiles existed and was available during accretion of solids. There is some evidence that in ordinary chondrites Pb is not highly concentrated in the sulphide^{23,24}, as would be expected for equilibrium condensation from a gas with free access to sulphide grains. Furthermore, the existence of troilite in a high temperature mineral assemblage indicates that thermodynamic modelling of condensation must be extended to embrace non-solar compositions of gas. Also, a purely hydrogen atmosphere⁸ is probably inappropriate for experimental heating of chondrites.

Finally, the presence of dark rims around norite clasts indicates that at least one period of fractionation preceded the accretion of Tieschitz. Perhaps ordinary chondrites are not really primitive objects, or maybe depletion in hydrogen occurred due to kinetic effects in the atmosphere of a growing planet which had received material from the break-up of an already differentiated body. This argument has already been put forward²⁵.

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Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex

Charles D. Gilbert & Torsten N. Wiesel

Department of Neurobiology, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115

The neuronal structure and connectivity underlying receptive field organisation of cells in the cat visual cortex have been investigated. Intracellular recordings were made using a micropipette filled with a histochemical marker, which was injected into the cells after their receptive fields had been characterised. This allowed visualisation of the dendritic and axonal arborisations of functionally identified neurones

CONSIDERABLE insight into the stages of visual information processing has been obtained from analysis of single cell responses to patterned light stimulation. This approach revealed that cells in the visual cortex respond optimally to specific stimuli, such as line segments of given orientation and length^{1,2}, and that neurones differ in their specificity. Cortical neurones also show great morphological diversity³⁻⁷, suggesting that there may be a correlation between the function of a cell and its

structure. Another feature of cortical organisation is that cells are segregated into layers, and in each layer the cells have distinct size, shape and packing density. This lamination has long been thought to be important in cortical function⁸⁻¹⁰. The layers differ further in the receptive field properties of the cells within them^{2,11-13}, in the inputs that they receive¹⁴⁻¹⁷ and in the sites to which their cells project¹⁸⁻²². This information was derived from extracellular recording and anatomical tracing, but now intracellular marking techniques, first used in the visual cortex by Kelly and Van Essen²³, can extend these findings by showing the detailed intracortical wiring of cells with known response properties.

In this study we have been able to trace the intracortical projections of functionally identified neurones in different layers by intracellular injection of the enzyme horseradish peroxidase (HRP)^{24,25}. Comparison of the receptive field properties of marked cells with those of cells in the layers to which they project provides evidence for the manner in which receptive fields are constructed.

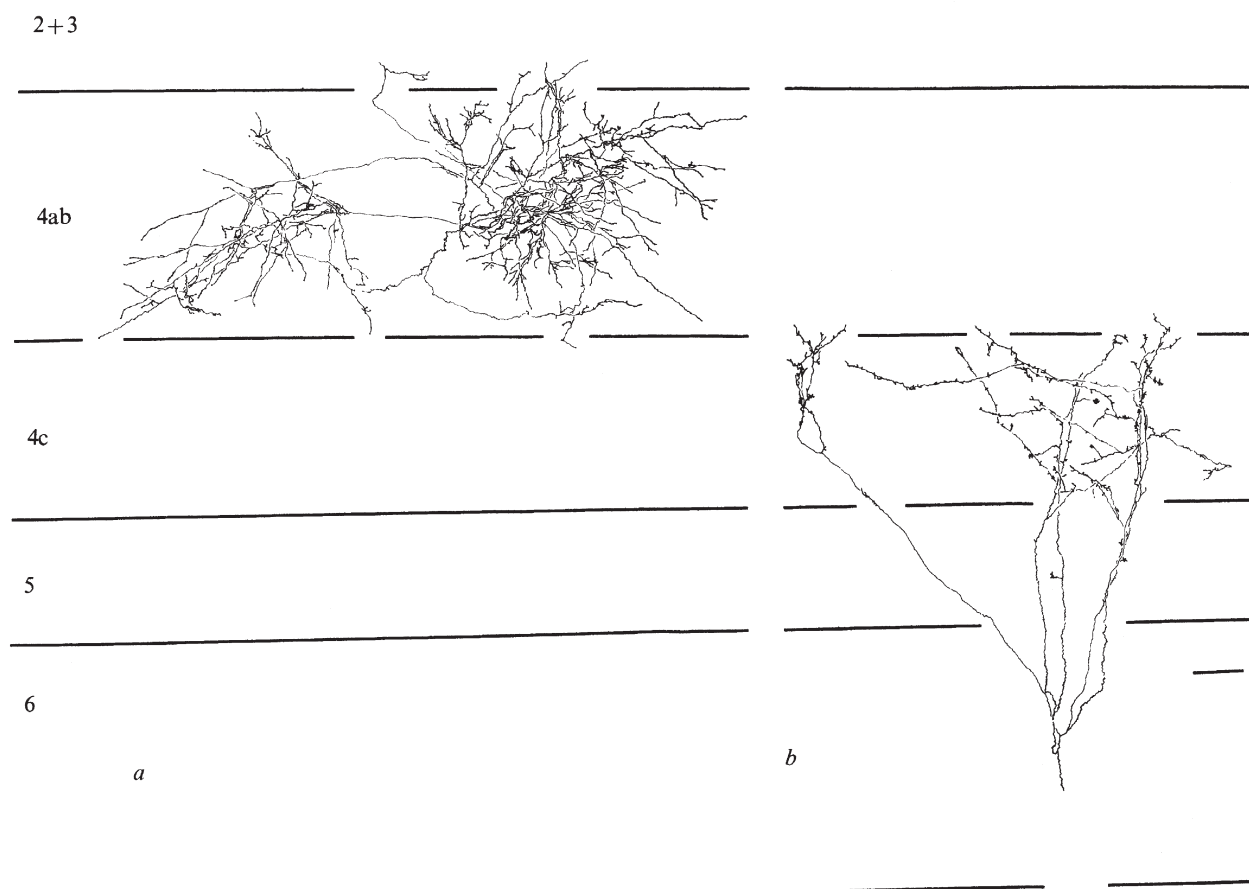


Fig. 1 Two afferents from the lateral geniculate nucleus, injected within the cortex. Was an off-centre Y-cell (centre size 1.5°, located 5° from the area centralis), and ramified entirely within layer 4ab. Was an off-centre X-cell (centre size 1°, located 3° from the area centralis), which ramified entirely within layer 4c. Scale bar, 100 μ m.

Table 1 Numbers and laminar positions of injected cells

Layer	No. of cells
2 + 3	16
4ab	4
4c	2
5	19
6	11
Afferents	9
Total %	61

Functional characterisation and labelling of visual cortical neurones

Recordings were made in cats maintained on sodium thiopental anaesthesia, paralysed with succinylcholine and artificially respirated. The animals' electrocardiogram, electroencephalogram, temperature and expired CO₂ concentration were monitored. A small hole was drilled in the skull above the visual cortex, and the dura and pia were opened. Half-micrometre bevelled-tip electrodes were filled with a solution of 4% HRP (Boehringer-Mannheim, grade I) in 0.2 M KAc, pH 7.6. After penetration of the brain surface with the electrode, the hole in the skull was filled with agar to reduce pulsations. The electrodes were advanced through the brain with a stepping microdrive (Transvertex) until a cell or process was penetrated, as indicated by a sudden change in the resting potential, the appearance of large action potentials and often synaptic activity. The receptive field properties of the cells were determined using either a hand-held projector or an optical bench.

The cells were injected with HRP either by pulses of pressure ranging from 0.5 to 5 atm or by pulses of positive current ranging from 1 to 2 nA in a 4 ms-on/4 ms-off duty cycle. At the end of the experiment each animal was perfused with a short buffer rinse followed by a long perfusion with 2% glutaraldehyde in the same buffer. After the brain was blocked, 150- μ m coronal sections were cut on a Vibratome (Oxford Instruments), and then treated with a combination of the diaminobutyric acid reaction at acid pH²⁶ and cobalt intensification²⁷. The injected cells were reconstructed from serial sections by using a microscope equipped with a drawing tube. Subsequently, the sections were counterstained with cresyl violet, and the laminar position of cells and their processes were determined using the criteria of Otsuka and Hassler²⁸. Table 1 lists the cells in each layer that we have injected to date.

Cortical afferents

Different morphological and physiological classes of retinal ganglion and geniculate cells are known to exist²⁹⁻³². Chief among these are the X- and Y-cells. These are defined by the linearity of spatial summation within their receptive fields²⁹, and also have been found to differ in their firing properties and axon conduction velocities^{30,33,34}. The dorsal geniculate laminae, which contain a mixture of X- and Y-cells³⁵, project to layers 4 and 6 in the visual cortex^{16,17}.

In this study we were able to penetrate and inject individual axons coming from the lateral geniculate nucleus either before or after they enter the cortex. We used the criteria of spatial summation to classify X- and Y-afferents^{29,36}. A reconstruction of each type is shown in Fig. 1. The Y-afferent (Fig. 1a) had off-centre/on-surround organisation, showed nonlinear summation, had a relatively large field centre (1.5°) and gave a transient response to a stationary flashing spot of light. It ended in layer 4ab in a rich arbor distributed in two patches separated by a terminal-free gap. The patches presumably correspond to ocular dominance columns driven by one eye, and the intervening gap to the column driven by the opposite eye^{15,37}. Some of the injected Y-afferents also sent collaterals to the upper half of layer 6.

In contrast to the afferents showing nonlinear summation, the X-afferent arborised entirely within layer 4c (Fig. 1b). No afferents were found to arborise in both sublayers of layer 4. This demonstrates, in agreement with Ferster and Levay³⁸, that the dorsal geniculate layers, which contain a mixture of at least two principal cell types, keep the input from the two types segregated on their arrival in the cortex.

Layer 4 cells

Cells in layer 4 lie in the terminal field of the geniculate afferents, and consequently represent the first level of processing in the cortex. Extracellular recordings show that the overwhelming majority of the cells in layer 4 have simple receptive fields^{1,11,23}, as defined by Hubel and T.N.W.³⁷. In addition to their specificity for stimulus orientation, simple cells can show a reduction in response to slits longer than the receptive field^{11,39,40}. This property, known as end-inhibition, makes cells optimally responsive to orientated lines of a defined length. Figure 2a shows an example of a simple cell in layer 4ab. It was a spiny stellate cell; the axon branched several times soon after leaving the soma, with a number of collaterals innervating in layer 4ab and then giving off a rich terminal arborisation in layer 2 + 3. The axon proceeded out of the cortex into the white matter, sending a few collaterals into the lower layers in its downward course. This general projection pattern was seen for all injected 4ab spiny cells and has also been observed with Golgi stains³⁻⁶. The horizontal extent of the axonal arborisation was much larger than that of the dendritic arborisation. This divergence could produce a further mixing in the input from the two eyes onto layer 2 + 3 cells, and could account for the higher proportion of binocularly driven cells found in that layer than in layer 4 (refs 1, 11).

Only two cells have been injected in layer 4c, one a small spiny stellate cell and the other a smooth stellate cell (Fig. 2b). They had very similar receptive field properties; both had simple receptive fields with on-centres, which were much smaller than those of the layer 4ab simple cells. The smooth stellate cell's axon ramified extensively throughout layer 4. The axon of the spiny stellate cell gave off many collaterals, some remaining within layer 4c and others extending to layer 4ab. Although the cell's axon was restricted to layer 4 (which is consistent with Golgi findings⁵), the existence of complex cells with very small receptive fields in layer 2 + 3 (refs 1, 11) leads us to expect that there should be other layer 4c cells with projections to that layer.

Layer 2 + 3 cells

The predominant intracortical projection from layer 4 seems to be to layer 2 + 3, which then represents the second level of cortical processing. Layer 2 + 3 almost exclusively contains complex cells^{1,11}. They are orientation selective, but differ from simple cells in having uniform receptive fields not divisible into separate on- and off-subregions and in responding continuously as a slit of light is moved across their fields^{1,11,41}. Like simple cells, they can be directional and end-inhibited. For complex cells, specificity for orientation and length is maintained, but compared with simple cells they have gained some freedom in the precise position of the stimulus along the movement axis¹.

A complex cell with a small receptive field (1° × 1°), showing no end-inhibition, is shown in Fig. 3. It was a pyramidal cell located in the upper part of layer 2 + 3. The basal dendrites ramified close to the soma and the apical dendrite extended into the lower part of layer 1. Its axon branched richly in its layer of origin, both in a region adjacent to the cell's dendritic field and in a region rather more distant, perhaps to a column of cells with the same orientation preference. The axon collaterals also extended into layer 1. The descending axon projected extensively within layer 5; this was a common feature of pyramidal cells in this layer, in agreement with degeneration⁴²⁻⁴⁴ and Golgi^{4,5,45} studies. The axon proceeded out of the cortex, presumably to innervate another cortical area or areas²¹.

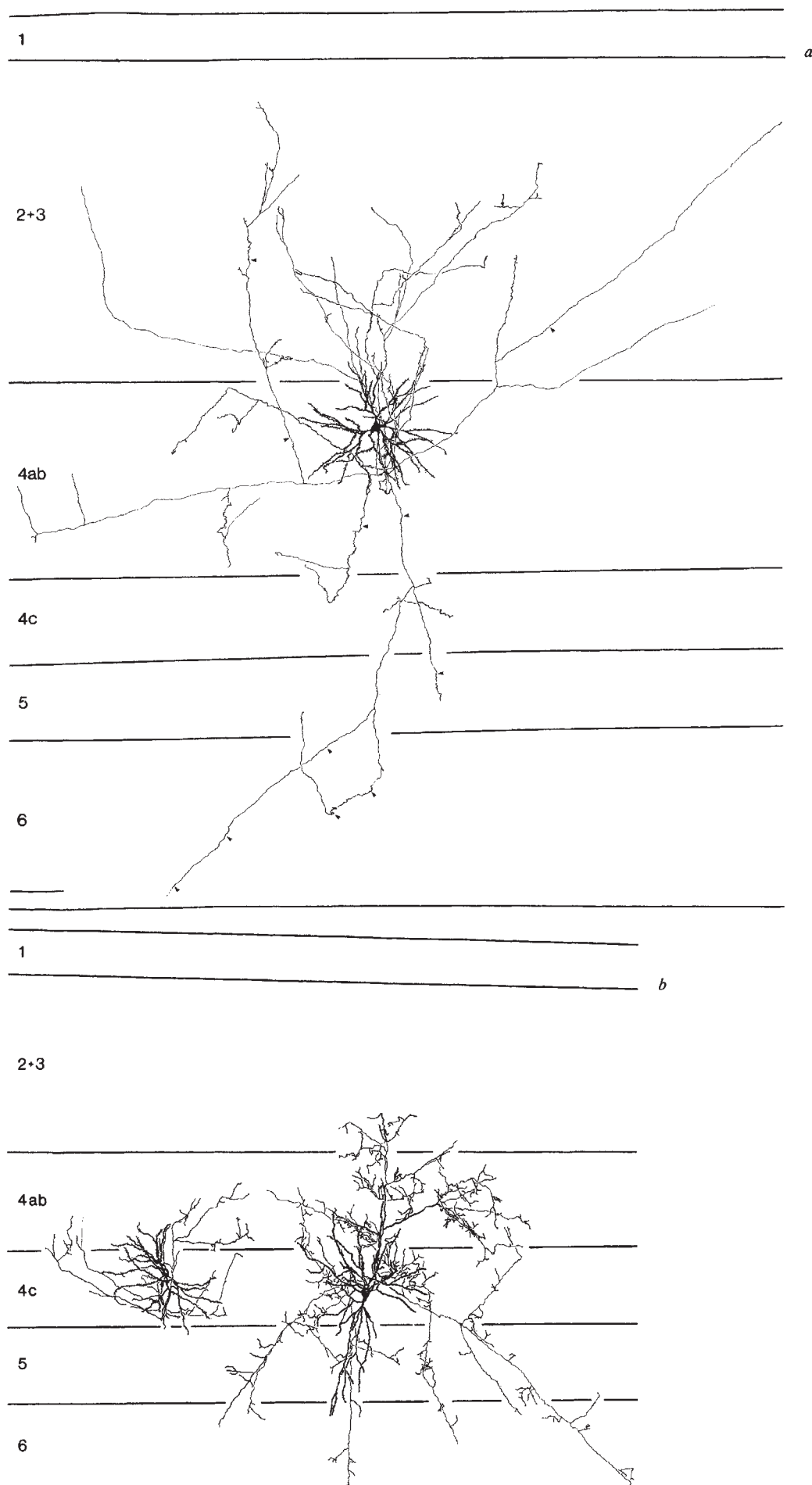


Fig. 2 *a*, A spiny stellate cell in layer 4ab having a simple receptive field with an on-centre and off-flanks, and showing no end-inhibition. The field size was $3^{\circ} \times 4^{\circ}$, and it was located 10° from the area centralis. The arrows indicate the positions of nodes of Ranvier. *b*, A spiny stellate cell (left) and a smooth stellate cell (right) in layer 4c. Both had simple receptive fields with on-centre and off-flanks, the spiny stellate cell showing no end-inhibition and the smooth stellate cell showing 50% end-inhibition. The size of the central on-portion of the field for both cells was $1^{\circ} \times \frac{1}{2}^{\circ}$, and both were located 2° from the area centralis. Because the plane of section was not perpendicular to the layers, this reconstruction makes it seem as if several sets of collaterals of the smooth stellate cell extend into layers 5 and 6, whereas in fact they remain within 4c. Scale bar, 100 μm .

Layer 5 cells

Cells in layer 5 have complex receptive fields like those in layer 2+3, but differ most notably in their large field sizes. Previous studies^{11,20} have shown that within layer 5 two cell types can be distinguished: one that shows summation for increased slit length (the standard complex cell) and one that responds optimally to a small moving slit of light placed anywhere within its relatively large field, showing no summation for increased slit length (the special complex cell). Consequently, the special complex cell maintains specificity for orientation, length and direction of stimulus movement, but gains some freedom in the precise position of the stimulus along the orientation axis. Like simple and standard complex cells, the special complex cell can exhibit end-inhibition¹¹.

Figure 4 shows a standard complex cell in layer 5. A surprising finding was that the cell's axon sent an extensive projection to layer 6, passing 6–8 mm down the medial bank, still remaining within area 17. The cell's receptive field was $2\frac{3}{4}^{\circ} \times 1\frac{3}{4}^{\circ}$, and its axon spanned an area of cortex representing up to 15° of visual field. Thus, it reaches areas that deal with parts of the field of view far outside the cell's receptive field. This projection has not been seen with Golgi stains, possibly because the axon becomes myelinated soon after leaving the cell body, and would therefore not impregnate with the Golgi technique. The distribution of the axonal field as viewed from the cortical surface was long and narrow. One might expect that the overall axis of distribution of the axon would be related to the orientation axis of the cell's receptive field. The axonal distribution and orientation of this cell were consistent with this view. The apical dendrite extended up into layer 1, branching repeatedly there, and several of its processes passed just underneath the pia. The cell had a dense dendritic arbor near its cell body, with a number of processes leaving the base of the apical dendrite at the layer 4/5 border, and a set of basal dendrites extending down into the upper part of layer 6.

We have also injected special complex cells in layer 5. Their axons seemed not to project as richly to layer 6 as those of the standard complex cells, and set a large trunk out of the cortex, presumably to innervate the superior colliculus^{20,21}.

Layer 6 cells

Layer 6 is of special interest as it receives a direct projection from the lateral geniculate nucleus¹¹ and contains a mixture of simple and complex cells^{1,11,23}. Its cells have unique receptive field properties, often requiring long slits for activation and showing summation with slit length up to very large values (16°)¹¹. Figure 5 shows an example of a layer 6 simple cell. The basal dendrite of the cell branched in the upper part of the layer; this is interesting as the collaterals of geniculate axons in layer 6 end in the upper part of the layer (see ref. 38 and also above). Its axon projected mainly to layer 4ab, in the vicinity of its apical dendrite, which branched extensively and ended in 4ab. Other cells, both pyramidal and smooth stellate, had axonal fields that lay almost exclusively within layer 6.

Although the simple cells of layer 6 were restricted to the upper half of the layer, we found complex cells throughout the layer. The apical dendrites of these cells branched at different levels within the cortex, which is reminiscent of the pattern observed in the monkey by Lund and Boothe⁴⁵. Like the cell in Fig. 5, they often sent collaterals primarily to layer 4, and these were restricted to either 4ab or 4c. The injected smooth stellate cell had a complex receptive field.

Intracortical pathways and receptive field construction

Although we have not yet injected all the morphological cell types found in the cortex⁴, some patterns of projection have

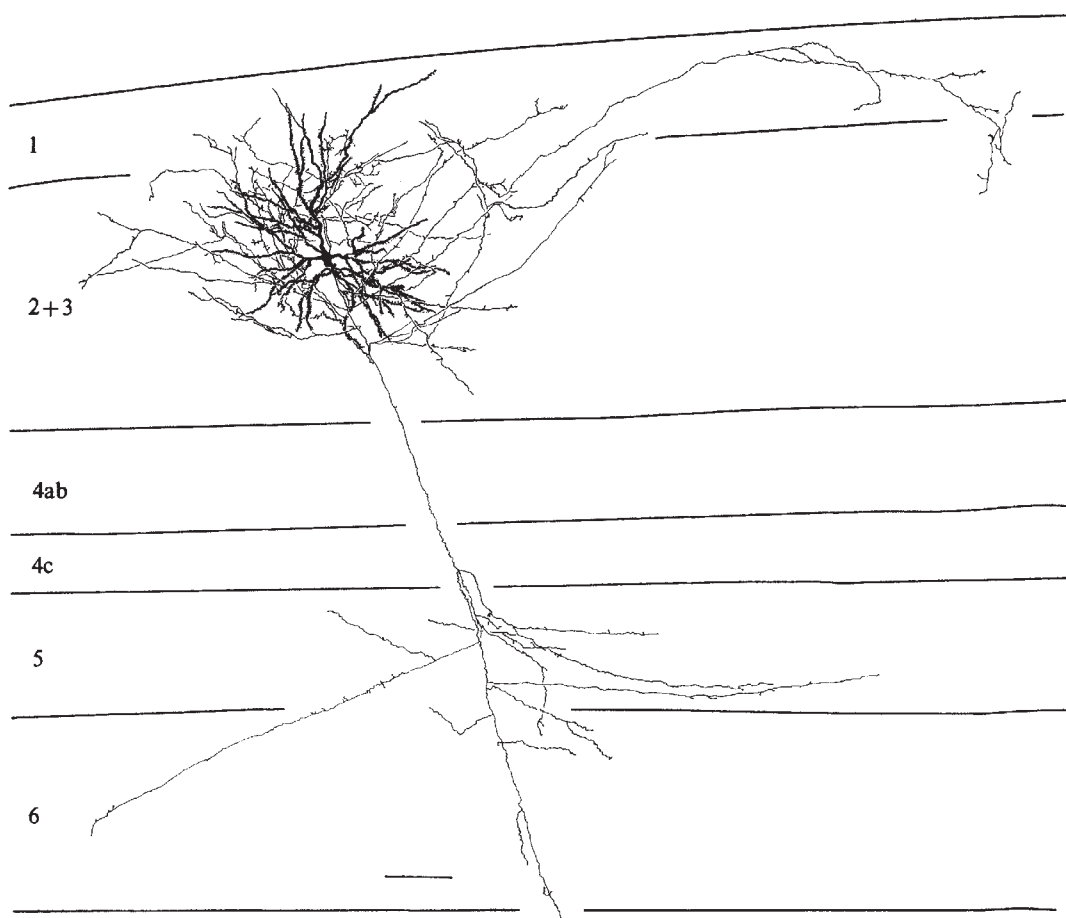


Fig. 3 A pyramidal cell in the upper part of layer 2+3, with a complex receptive field showing no end-inhibition. The field size was $1^{\circ} \times 1^{\circ}$, and it was located 4° from the area centralis. Scale bar, 100 μm .

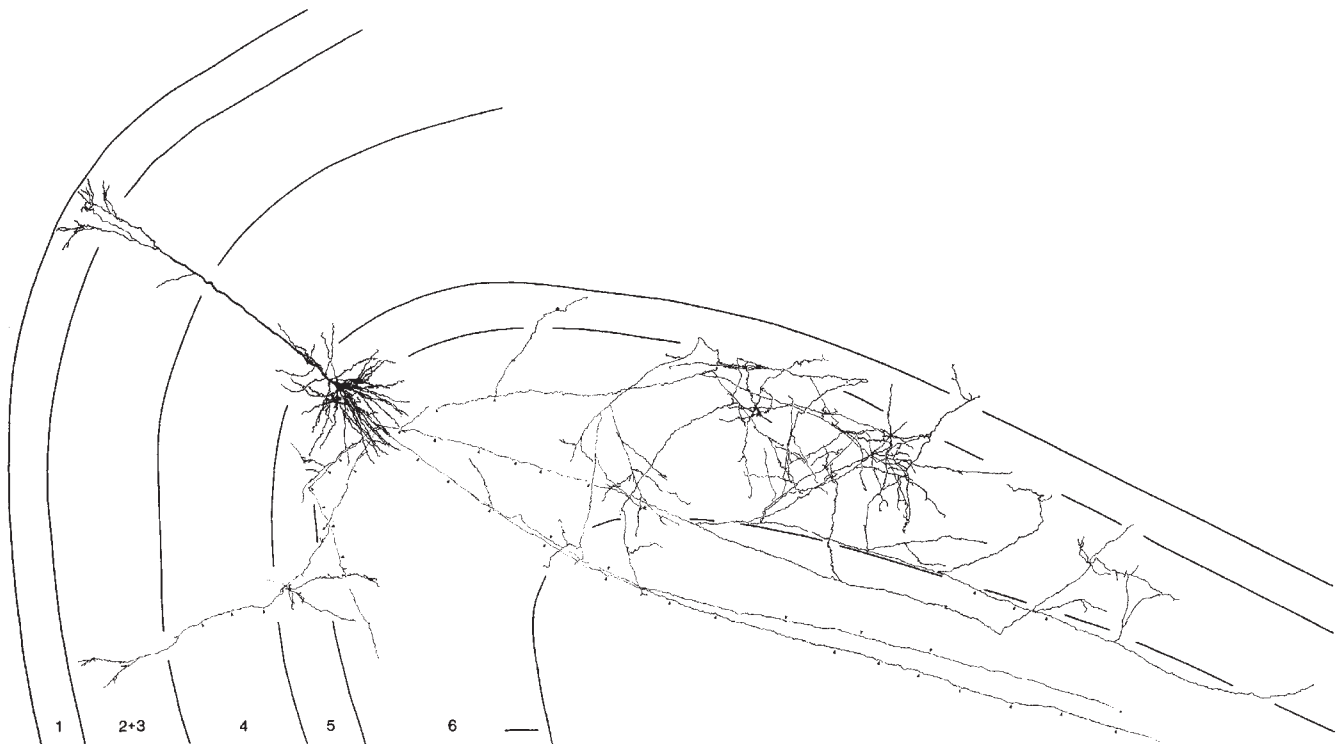


Fig. 4 A pyramidal cell in layer 5, with a standard complex receptive field showing no end-inhibition. The field size was $2.4^{\circ} \times 1.4^{\circ}$, and it was located on the area centralis. The branches of the apical dendrite within layer 1 all run quite close to the pia, but this is not apparent due to the plane of the section relative to the cortical layers. Scale bar, 100 μm .

emerged from our present catalogue of injected cells. The predominant stages of information processing in the cortex seem to be as follows: the major geniculate input to the cortex arrives in layer 4, with the X-input and the Y-input arriving separately in layers 4c and 4ab. Both inputs also project to the upper half of layer 6. From layer 4 the sequence of processing continues to the upper layers, from the upper layers to layer 5, and from layer 5 to layer 6. Layer 6 then sends output back into layer 4. The cortex is tapped for output to other regions at several stages: cortical areas from layer 2 + 3, superior colliculus from layer 5, and lateral geniculate nucleus from layer 6. Also, the spread seen in the horizontal axonal projections (Fig. 4) extends far beyond that expected from previous studies using the Golgi technique³⁻⁶.

This pattern enabled us to form hypotheses as to the manner in which receptive fields are constructed within the cortex. As originally suggested by Hubel and T.N.W.⁴⁶, simple receptive fields are constructed from the fields of lateral geniculate neurones, as simple cells are the predominant class in layer 4, the major geniculate afferent zone. The correspondence between simple receptive field type and geniculate input is also seen for the cells that lie in the upper part of layer 6. Although they share the same general receptive field type, cells in layer 4c have smaller fields than those in 4ab. This difference is consistent with the two features of the geniculate input to layer 4—the X-afferents have smaller receptive fields than the Y-afferents, and the 4ab afferents have a much wider terminal arborisation than the 4c afferents³⁸. Although the receptive fields of the injected 4c cells were quite similar, spiny cells are thought to be excitatory and smooth cells inhibitory. These presumptions are based on both their synaptic morphology and transmitter neurochemistry⁴⁷⁻⁴⁹.

The superficial layer complex cells receive their input from the layer 4 simple cells. As cells in layer 4ab project to layer 2 + 3, and as, except for the bottom of layer 3, the geniculate does not project to this layer, it is likely that the receptive fields of cells in the upper layers are generated primarily by input from layer 4.

The layer 5 complex cells may form their fields from the concatenation of the fields of the superficial complex cells, as suggested by the extensive projection from the layer 2 + 3 cells into layer 5. The lateral spread of this projection can account for the increase in receptive field size from the superficial layers to layer 5. Because their apical dendrites pass through geniculate afferent zones, the layer 5 complex cells may also receive geniculate input. Evidence from serial electron microscopic reconstructions suggests, however, that they do not⁵⁰. We do not know what are the differences in the inputs to standard and special complex cells which account for their differences in receptive field properties.

The substantial horizontal traverse of the layer 5 axon in layer 6 is an intriguing feature, as the layer 6 cells have very long receptive fields, showing summation for increased slit length up to very large values, some reaching 16° or more¹¹. We have seen no other inputs to layer 6 or intrinsic connections within layer 6 that can account for this receptive field property; the input to layer 6 from the lateral geniculate nucleus is, if anything, more restricted than the geniculate input to layer 4 (refs 17, 38). Also, none of the cells within layer 6 that we have injected thus far have axons that ramify within the layer over nearly as large an area as do the axons from the layer 5 cells. This hypothesis requires us to account for elongation in both simple and complex fields in layer 6 despite the fact that the input from layer 5 is exclusively complex. The simple fields may be produced by an interaction between the geniculate input to the upper half of layer 6 and the input from layer 5.

The rich projection from layer 6 to layer 4 suggests that some properties manifested by layer 4 cells may not depend solely on convergence of geniculate input onto layer 4 cells or on connections made by those cells within the layer. It could be that, due to their intimate involvement with layer 4 through dendritic arborisation and axonal projection, the layer 6 cells have a role in producing orientation specificity, preference for direction of stimulus movement and/or end-inhibition.

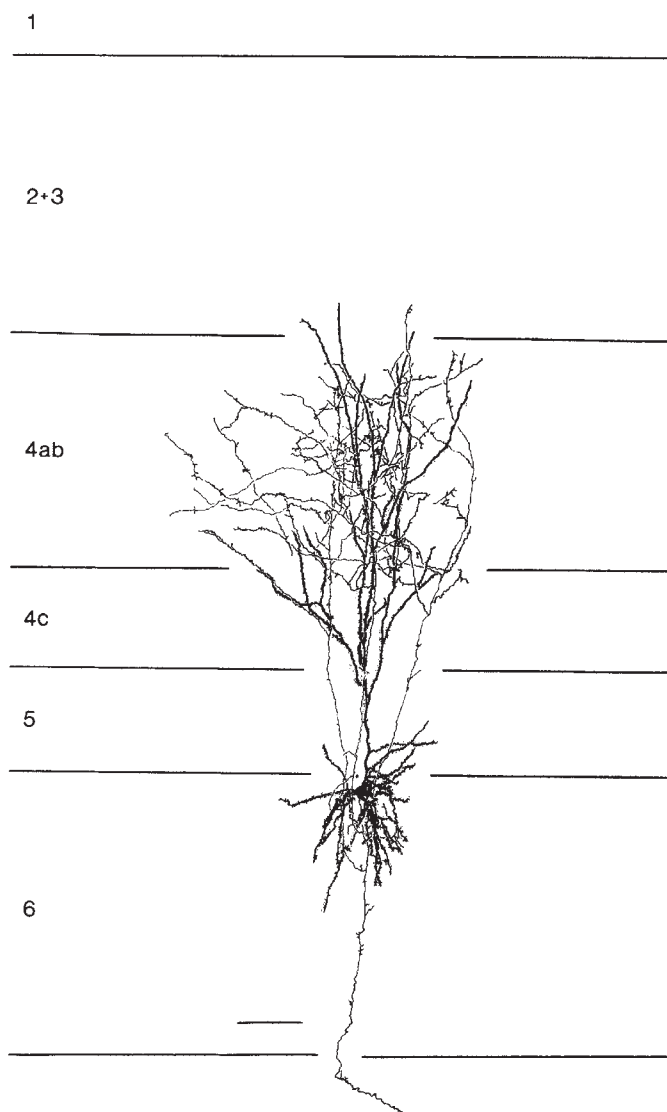


Fig. 5 A pyramidal cell in layer 6, with a simple receptive field showing the lack of end-inhibition characteristic of cells in this layer. The field size was $1\frac{1}{2} \times 2^\circ$, centred 4° from the area centralis. It did not show obvious summation to long slits and may represent a population of layer 6 cells with shorter fields. Scale bar, 100 μm .

Dendritic morphology and receptive field construction

Several issues are raised in considering correlations between morphology and function. As seen from our injections in layer 5, for example, large neurones have large receptive fields. This is presumably due to their large dendritic fields, which enable the cells to collect input over a wide area. Another determining factor in receptive field size is the extent of axonal ramification of the inputs to a given cell. This is seen for the layer 6 cells; although their dendritic fields are relatively small, they receive input from layer 5 over a very large area, due to the wide spread of the layer 5 cell axons. The layer 5 fields may derive their large area from both mechanisms: they have very wide dendritic arbors, and the layer 2 + 3 cells have widely arborising axons in layer 5.

A correlation between simple receptive field type and stellate morphology was found by Kelly and Van Essen²³, but, as they indicated, this was not a strict correlation. We have injected simple cells in layer 5a and 6 that were clearly pyramidal in morphology and one complex cell in layer 6 that was stellate. Thus, the simple/complex receptive field classification seems not to be the pertinent feature in relating functional properties

to the stellate/pyramidal categories. The determinant factor as to whether a pyramidal cell is simple or complex seems to be the position of its basal dendrites relative to the geniculate input. The function of apical dendrites remains unknown; they may ramify at different levels within the cortex, but so far this does not seem to correlate with any receptive field differences. It is possible that, as seen for some cells in layer 6, the role of the apical dendrite is to participate in the formation of recurrent loops within the cortex.

In addition to the dichotomy between stellate and pyramidal cells, there is, even for a given layer, considerable variability within each of these morphological classes. At this early stage in our study we do not know how this diversity is reflected in the receptive field and/or firing properties of cells. There are, on the other hand, certain receptive field features, such as orientation specificity and preference for direction of stimulus movement, which may correspond to particular aspects of dendritic arborisation. Another functional difference between cells is their inhibitory or excitatory postsynaptic effects, which depend on the transmitters they use. As mentioned above, this may be reflected in morphological differences and not in differences in receptive field properties. Pharmacological evidence suggests that inhibition is important in enhancing certain receptive field features, such as orientation specificity⁵¹. The relationship between function and dendritic morphology remains a challenge. We expect that further analysis of and additions to our catalogue of injected cells will reveal clues to this relationship.

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letters

The 78.4-day period of Cygnus XR-1

CYGNUS XR-1/HDE226868 is a multiple star system in which the secondary is the best astronomical candidate for a black hole. The existence of a third body in the system could affect the mass derived for the secondary from the analysis of spectroscopic radial velocity curves¹. Hence, the presence of a periodicity in the system other than that of the 5.6-d binary orbit has a major bearing on the identification of the secondary with a black hole, especially if such a periodicity can be related to the orbital period of a third body. The existence of a 78.4 (or 39.2)-d periodicity in the optical and low-energy X-ray wavelength region of the emission from this system has been reported by Kemp *et al.*^{2,3}. The existence of a 39.2-d modulation in the optical and low-energy X-ray luminosities has been questioned by Walker *et al.*⁴. We report here a search for a 78.4-d modulation in the high-energy X-ray flux observed with OSO 8 and in the U-band optical polarisation previously observed⁵, with negative results. We suggest, however, that if such modulation does exist, it is more likely to be related to the rotation of the free modes of oscillation of the primary than to the existence of a third body in the system.

Analysis of the power spectrum of the U-band polarisation of HDE226868 on 225 nights between 1974 and 1977 led to the original detection by Kemp *et al.*² of a 39.2-d modulation in the magnitude of polarisation with peak-to-peak amplitude of 0.25% (or 0.0054 mag). A modulation with a 78.4-d period was detected by Kemp *et al.*³ in the power spectrum analysis of the U-band photometry of the system taken during 43 nights in 1974 and 84 nights in 1977. The peak-to-peak modulation was approximately 0.015 mag. A reanalysis of the UV filter polarimetry at the 78.4-d period produced a double-peaked modulation; the 0.0054 mag modulation in the polarisation was equivalent to a peak-to-peak amplitude of approximately 8% of the mean polarisation. In a similar analysis of the power spectrum of the X-ray flux in the energy range 3–6 keV from Cyg XR-1 observed between October 1974 and December 1977 with the all-sky monitor on Ariel 5, Kemp *et al.*³ detected a 78.4-d modulation with peak-to-peak amplitude of approximately 15% of the mean flux. The modulation seems to be in phase with the U-band photometric modulation. This periodic modulation was detected only when the X-ray source was in an intensity 'low state'.

Cyg XR-1 was observed with the high energy X-ray spectrometer⁶ on OSO 8 during periods of 10–20 d in each of the years between 1975 and 1977 (ref. 7). The source exhibited a transition from the intensity low state to the intensity high state during the observation in 1975 (ref. 8). Consequently, only the data obtained in 1976 and 1977, when the source was continuously in a low state, were analysed for a 78.4-d modulation. The counting rates from the source between 23 and 153 keV, binned in intervals of one-tenth the 5.5995-d binary period, were folded modulo the 78.4-d period with epoch of zero phase as given by Kemp *et al.*³ (Fig. 1a). The weighted mean counting rate over intervals of 0.1 in phase are shown in Fig. 1b for direct comparison with the 3 to 6 keV X-ray data of Kemp *et al.* (Fig. 1c). The modulation observed by Kemp *et al.* at lower energies is not immediately apparent in our data. The variations evident in Fig. 1b have a peak-to-peak range of 19% of the mean flux between 23 and 153 keV. If any 78.4 (or 39.2)-d modulation

exists in the high-energy X-ray flux from Cyg XR-1, it must be at an amplitude significantly lower than this. Because of the irregular spacing of the data and the fact that all observing runs were short compared with the 78.4-d period, no more stringent limits could be placed on the existence of a 78.4-d modulation by the use of power spectrum analysis.

Observations of the polarisation of HDE226868 in the U-band were obtained over 23 nights in 1973, 1974 and 1975 (ref. 5). The individual observations were intended to investigate short time-scale phenomena: each U-band polarisation measurement was completed in 10 min. The observations were folded modulo the 78.4-d period with the same epoch of zero

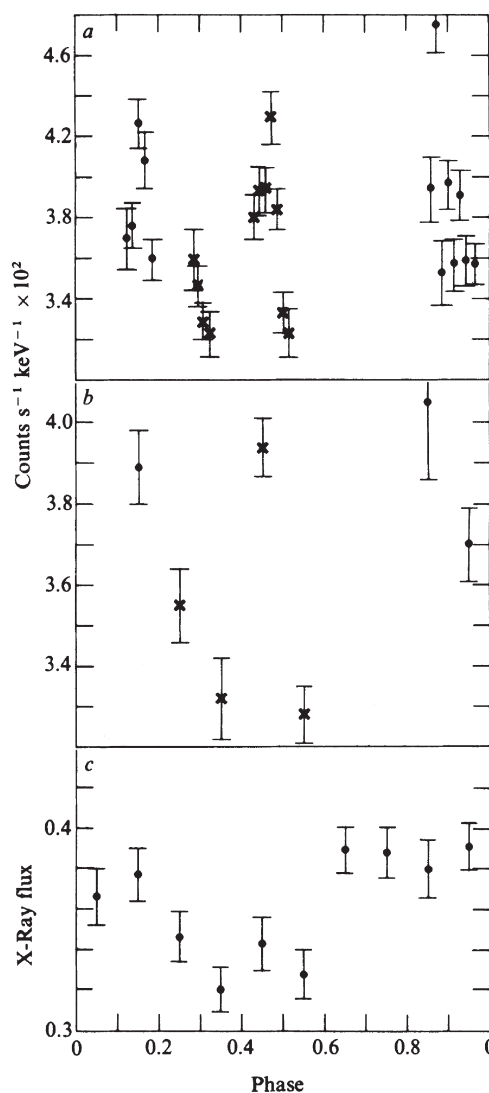


Fig. 1 X-ray counting rates observed from Cyg XR-1 plotted modulo the 78.4-d period of Kemp *et al.*³. (a), Individual observations of the 23–153 keV counting rates taken in 1976 (x) and 1977 (●). (b), Weighted mean counting rate of the observations in (a) over intervals of 0.1 in phase. (c), The 3–6 keV flux observed by Kemp *et al.*³.

phase as above. The weighted mean polarisation in intervals of 0.1 in phase shows variability with a peak-to-peak amplitude of 34% of the mean polarisation. Variations typically of this magnitude have been ascribed⁵ to Rayleigh scattering of light from intercomponent gas streams known to exist in the system. A χ^2 test shows that the data points are equally consistent with a random distribution about either a non-varying polarisation or the type of curve fitted to their data by Kemp *et al.*³. The data do place an upper limit on the existence, during our observations, of any variability of the type seen by Kemp *et al.*, but only at an amplitude four times that seen by them. Once again, the irregular spacing and greatly varying statistical weights of the individual observations preclude using a power spectrum analysis to set more stringent limits on any periodic modulation.

Kemp *et al.*³ propose a physical interpretation of a 78.4-d period in terms of either a ternary system, the precession of an accretion disk, or some kind of pulsation. They discuss the three-body possibility, although they point out that their analysis of spectroscopic radial velocity observations of the visible primary limits any third body in the system with a 78.4-d period to a mass of less than $(0.8 \pm 0.6) M_{\odot}$. We believe that a more likely interpretation of a 78.4-d periodicity in the system, if confirmed, is related to the fundamental rotation properties of the free modes of oscillation of the primary. In the Wolff and Kondo model^{9,10} of X-ray binary systems, for example, the non-degenerate star pulsates in a broad array of gravitational modes (*g*-modes), global in scale and lacking spherical symmetry. Under slow rotation and sufficient non-linear coupling, the *g*-modes can combine to form a much smaller set of non-linear modes departing from azimuthal symmetry. As the main antinodes of these non-linear modes rotate past each other, the oscillatory power density at these locations is temporarily enhanced. The occurrence of transitions between intensity states of the X-ray source is ascribed by Dolan *et al.*⁷ to sudden changes in the amount of mass transferred whenever the main antinodes are aligned with the sub-secondary point on the surface of the primary. In this picture, the 78.4-d period would be the time between effective alignments of certain *g*-modes.

As noted by Kemp *et al.*³, excess mass transfer between the binary components modulated with a 78.4 d period could explain all the modulation they see in the X-ray and UV emission. The continued existence of this period over 3 years implies that it may be maintained by some forcing function. If the 78.4-d period is an exact integral multiple of the 5.6-d orbital period of the secondary^{2,3}, tidal forces occurring every periastron passage of the secondary in its eccentric orbit ($e = 0.06$) could provide such a forcing function (see ref. 11). Further observations are necessary to determine whether the 78.4-d modulation can be confirmed, and whether it can be related to the rotation properties of the primary's free modes of oscillation.

M.J.C. is a NAS-NRC Resident Research Associate.

J. F. DOLAN
P. CARAVEO*
M. J. COE
C. J. CRANNELL
B. R. DENNIS
K. J. FROST
L. E. ORWIG

Laboratory for Astronomy and Solar Physics,
NASA/Goddard Space Flight Center,
Greenbelt, Maryland 20771

Received 1 February; accepted 30 May 1979.

* Permanent address: L.F.C.T.R., Via Bassini 15, 20133 Milano, Italy.

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Simultaneous effects of CO₂ and chlorofluoromethanes on stratospheric ozone

THE amount of ozone in the stratosphere is determined by a dynamic balance between processes from three disciplines: photochemical kinetics, radiative transfer and dynamical meteorology. These processes are interactively coupled, principally through the temperature of the air molecules; because of the low pressure and solar UV light a chain reaction is possible by which the composition of the stratosphere, and particularly its ozone content, may be affected by comparatively small quantities of trace species. Calculations have indicated that oxides of nitrogen from high-flying aircraft and nuclear weapons, nitrous oxide from artificial fertilisers and various chlorine-containing derivatives of methane and ethane (mainly CF₂Cl₂, CFC1₃, CCl₄ and CH₃CCl₃) could be large enough to act in this way. Reductions in ozone column density could lead to increases in the amount of solar UV light transmitted to the planetary surface in the wavelength region 290–320 nm, with possible effects on human health and biological activity. We have already shown that projected increases in carbon dioxide amounts in the atmosphere would lead to cooling and thus to ozone enhancement in the stratosphere, particularly at the upper levels. Here we extend these calculations to include simultaneous perturbations of CO₂ and chlorofluoromethanes.

The photochemical-radiative column model is as used previously^{1,2}, but it incorporates the following modifications. The solar radiative transfer scheme for the computation of photo-dissociation coefficients has been reformulated to include the effects of multiple scattering, in a manner similar to that of Isaksen *et al.*³. The chemical kinetic scheme has been expanded

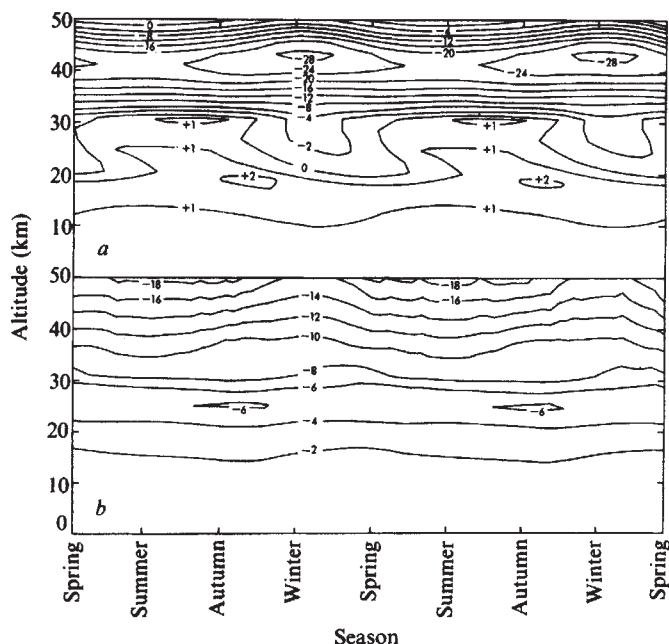


Fig. 1 Integration 5 minus integration 1 from Table 3. *a*, Percentage change in ozone number density; *b*, change in temperature (K).

Table 1 Photochemical kinetic mechanism and rate coefficients

Reaction	Mechanism	Rate coefficients (cm mol ⁻¹ s ⁻¹)	Reaction	Mechanism	Rate coefficients (cm mol ⁻¹ s ⁻¹)
1	O ₂ + hν → O + O		36	O(¹ D) + CH ₄ → OH + CH ₃	1.4 × 10 ⁻¹⁰
2	O ₃ + hν → O(¹ D) + O ₂		37	OH + O ₃ → HO ₂ + O ₂	1.5 × 10 ⁻¹² exp (-1,000/T)
3	O ₃ + hν → O + O ₂		38	O + HO ₂ → OH + O ₂	3.5 × 10 ⁻¹¹
4	N ₂ O + hν → O(¹ D) + N ₂		39	O + OH → O ₂ + H	4.2 × 10 ⁻¹¹
5	NO + hν → N + O		40	H + O ₃ → OH + O ₂	2.6 × 10 ⁻¹¹
6	NO ₂ + hν → NO + O		41	H + O ₂ + M → HO ₂ + M	2.1 × 10 ⁻³² exp (290/T)
7	HNO ₃ + hν → OH + NO ₂		42	HO ₂ + O ₃ → OH + O ₂ + O ₂	1.4 × 10 ⁻¹⁴ exp (-580/T)
8	NO ₃ + hν → NO ₂ + O		43	OH + HO ₂ → H ₂ O + O ₂	5.1 × 10 ⁻¹¹
9	N ₂ O ₅ + hν → NO ₂ + NO ₃		44	HO ₂ + HO ₂ → H ₂ O ₂ + O ₂	6.4 × 10 ⁻¹³ exp (500/T)
10	HO ₂ + hν → OH + O		45	OH + H ₂ O ₂ → HO ₂ + H ₂ O	1.0 × 10 ⁻¹¹ exp (-750/T)
11	H ₂ O ₂ + hν → OH + OH		46	OH + CH ₄ → H ₂ O + CH ₃	2.4 × 10 ⁻¹² exp (-1,710/T)
12	HCl + hν → H + Cl		47	OH + NO ₂ → HNO ₃	k ₄₇ = UV[M]/(U + V[M]); U = (68 - 0.22T)10 ⁻¹² , V = 3.7 × 10 ⁻³² exp (1,093/T)
13	CH ₃ Cl + hν → CH ₃ + Cl		48	NO + HO ₂ → NO ₂ + OH	8.1 × 10 ⁻¹²
14	ClONO ₂ + hν → NO ₂ + ClO		49	OH + HNO ₃ → H ₂ O + NO ₃	8.0 × 10 ⁻¹⁴
15*	CF ₂ Cl ₂ + hν → 2Cl		50	OH + OH → H ₂ O + O	1.0 × 10 ⁻¹¹ exp (-550/T)
16*	CFCl ₃ + hν → 3Cl		51	OH + CO → (CO ₂) + H	(1.0 + 4.18 × 10 ⁻²⁰ [M]) × 2.1 × 10 ⁻¹³ exp (-115/T)
17*	CCl ₄ + hν → 4Cl		52	NO ₂ + HO ₂ + M → HNO ₄ + M	See Table 2
18	HNO ₄ + hν → HO ₂ + NO ₂		53	HNO ₄ + M → HO ₂ + NO ₂ + M	See Table 2
19	O + O ₂ + M → O ₃ + M	1.1 × 10 ⁻³⁴ exp (510/T)	54	Cl + O ₃ → ClO + O ₂	2.7 × 10 ⁻¹¹ exp (-257/T)
20	O + O ₂ → O ₃ + O ₂	1.9 × 10 ⁻¹¹ exp (-2,300/T)	55	O + ClO → Cl + O ₂	1.07 × 10 ⁻¹⁰ exp (-224/T)
21	O(¹ D) + M → O + M	2.0 × 10 ⁻¹¹ exp (107/T)	56	ClO + NO ₂ + M → ClONO ₂ + M	See Table 2
22	O(¹ D) + N ₂ O → NO + NO	5.5 × 10 ⁻¹¹	57	ClO + NO → NO ₂ + Cl	8.0 × 10 ⁻¹² exp (250/T)
23	O(¹ D) + N ₂ O → N ₂ + O ₂	5.5 × 10 ⁻¹¹	58	O + ClONO ₂ → ClO + NO ₂	4.5 × 10 ⁻¹² exp (-840/T)
24	NO + O ₃ → NO ₂ + O ₂	9.0 × 10 ⁻¹³ exp (-1,200/T)	59	Cl + CH ₄ → HCl + CH ₃	7.3 × 10 ⁻¹² exp (-1260/T)
25	NO ₂ + O → NO + O ₂	9.1 × 10 ⁻¹²	60*	OH + HCl → H ₂ O + Cl	3.1 × 10 ⁻¹² exp (-437/T)
26	NO ₂ + O ₃ → NO ₃ + O ₂	1.2 × 10 ⁻¹³ exp (-2,450/T)	61	Cl + HO ₂ → HCl + O ₂	2.5 × 10 ⁻¹¹
27	NO + NO ₃ → NO ₂ + NO ₂	8.7 × 10 ⁻¹²	62*	OH + CH ₃ Cl → H ₂ O + Cl	2.2 × 10 ⁻¹² exp (-1,142/T)
28	NO ₂ + NO ₃ → N ₂ O ₅	See Table 2.	63	O(¹ D) + CH ₃ Cl → ClO + CH ₃	1.8 × 10 ⁻¹⁰
29	OH + ClONO ₂ → NO ₃ + (HOCl)	1.2 × 10 ⁻¹² exp (-333/T)	64*	O(¹ D) + CF ₂ Cl ₂ → ClO + Cl	2.0 × 10 ⁻¹⁰
30	N + NO → N ₂ + O	8.2 × 10 ⁻¹¹ exp (-410/T)	65*	O(¹ D) + CFCl ₃ → ClO + 2Cl	2.3 × 10 ⁻¹⁰
31	N + NO ₂ → N ₂ O + O	2.0 × 10 ⁻¹¹ exp (-800/T)	66*	O(¹ D) + CCl ₄ → ClO + 3Cl	2.5 × 10 ⁻¹⁰
32	N + O ₂ → NO + O	5.5 × 10 ⁻¹² exp (-3,220/T)	67	O(¹ D) + HCl → OH + Cl	1.4 × 10 ⁻¹⁰
33	N + O ₃ → NO + O ₂	5.0 × 10 ⁻¹² exp (-650/T)	68	CH ₃ → 3HO ₂	Parameterisation; t _{CH₃} = 100 s
34	N ₂ O ₅ + M → NO ₂ + NO ₃	k ₂₈ × 5.7 × 10 ¹⁴ exp (-10,600/T)/3.8 × 10 ⁻¹²			
35	O(¹ D) + H ₂ O → OH + OH	2.3 × 10 ⁻¹⁰			

* In these reactions, effective products are shown. Bracketed product molecules are not carried in the calculation.

Table 2 Rate coefficients for pressure-dependent reactions

Altitude (km)	Reaction 28 (cm ³ mol ⁻¹ s ⁻¹)	Reaction 52 (cm ³ mol ⁻¹ s ⁻¹)	Reaction 53 (s ⁻¹)	Reaction 56 (cm ³ mol ⁻¹ s ⁻¹)
48	4.8 × 10 ⁻¹⁴	7.3 × 10 ⁻¹⁵	1.2 × 10 ⁻⁵	5.5 × 10 ⁻¹⁵
46	6.5	1.1 × 10 ⁻¹⁴	5.5 × 10 ⁻⁶	8.4
44	8.7 × 10 ⁻¹³	1.6	2.9	1.3 × 10 ⁻¹⁴
42	1.2	2.3	2.2	1.8
40	1.5	3.4	1.5	2.7
38	2.0	5.0	6.0 × 10 ⁻⁶	3.9
36	2.4	7.2	3.2	5.8
34	3.0	1.0 × 10 ⁻¹³	2.0	8.0
32	3.9	1.5	1.4	1.2 × 10 ⁻¹³
30	4.5	2.0	9.6 × 10 ⁻⁸	1.6
28	5.2	2.7	8.5	2.2
26	6.0	3.7	7.0	3.0
24	7.0	4.8	5.5	3.9
22	8.3	6.2	4.0	5.2
20	1.1 × 10 ⁻¹²	7.5	3.5	6.5
18	1.3	9.2	4.0	8.0
16	1.7	1.1 × 10 ⁻¹²	8.0	9.3
14	2.0	1.3	1.5 × 10 ⁻⁷	1.1 × 10 ⁻¹²
12	2.2	1.4	3.2	1.1
10	2.2	1.5	4.4	1.2
8	2.2	1.6	5.5 × 10 ⁻⁶	1.2
6	2.3	1.6	6.0 × 10 ⁻⁵	1.2
4	2.5	1.7	8.0 × 10 ⁻⁴	1.2
2	2.5	1.7	9.0 × 10 ⁻³	1.2
0	2.5	1.8	1.3 × 10 ⁻¹	1.3

Table 3 Mixing ratios of stable chlorine-containing species

Integration no.	CO ₂ (p.p.m.v.)	CH ₃ Cl (10 ¹⁰ v.m.r.)	CCl ₄ (10 ¹⁰ v.m.r.)	CFCl ₃ (10 ⁹ v.m.r.)	CF ₂ Cl ₂ (10 ⁹ v.m.r.)	% Change in ozone column density	Comments
1	275	7.5	1.25*	0	0	—	Reference atmosphere
2	600	7.5	1.25	0	0	+3.2	
3	600	7.5	1.25	0.8	2.3	-6.3	Steady state CFMs 1975 rates
4	275	7.5	1.25	0.8	2.3	-8.6	Steady state CFMs 1975 rates
5	600	7.5	1.25	0.4	1.15	-2.6	Approximately 2020 AD

* There may be both natural and man-made contributions to this species in the atmosphere⁵⁻⁷.

to that detailed in Tables 1 and 2. The lower boundary conditions are as before except for nitrous oxide, the mixing ratio of which is 3.25×10^{-7} , in line with recent measurements⁴. The mixing ratios of stable chlorine-containing species are listed in Table 3; the new soluble species such as HCl have been added to the list of molecules rained out. A series of five integrations has been made, as shown in Table 3. The design of our radiative transfer scheme does not allow realistic calculation of the effects of CO₂ increases much beyond a factor or two from 350 p.p.m.v. Therefore we have not attempted to simulate the case of simultaneous projected CO₂ (approximately 1,100 p.p.m.v.) and steady state CFM amounts at 1975 release rates, which may occur about the period 2060–2070. Instead, we have computed the effects of steady state CFM release with 600 p.p.m.v. of CO₂. Integration 5 may be viewed as a possibly realistic schedule for simultaneous CO₂ and CFM release, with the former at 600 p.p.m.v. and the latter at half their steady state values. This is projected to represent approximately the period 2020–2030. It is apparent that the net decrease in ozone column density computed for this time is 2.6%. Earlier integrations performed without chlorine chemistry in the model² yielded an increase of 5.5% when the CO₂ was allowed to rise from 275 p.p.m.v. to 600 p.p.m.v.; the extra 2.3% compared with integration 2 of Table 3 must thus be attributed to this absence of chlorine from

the model. The remaining integrations demonstrate that the effects of CO₂ and CFMs on ozone column density are not quite linearly additive. Given this, it is apparent that in the simulated period about 2020, the CO₂ at 600 p.p.m.v. substantially alleviates the reductions in stratospheric ozone caused by continued CFM release at 1975 rates. The result of integration 4 yields a mean annual reduction of 8.6% in the ozone column density from steady state CFMs, if no increase in CO₂ over that in the reference atmosphere occurs. This is substantially less than recent estimates of some other column models^{8,9}, which have given figures of 15–20%. Presumably a fully diurnal model with interactive radiative transfer is more stable towards chlorine perturbation.

The comparatively small reduction in O₃ column density predicted in integration 5 was achieved at the cost of a substantial redistribution as a function of altitude, with a concomitant change in vertical temperature structure, as shown in Fig. 1. The importance of the coupling between ozone, temperature and large scale stratospheric dynamics¹⁰ in this respect should be recalled.

K. S. GROVES
A. F. TUCK

Meteorological Office,
London Road, Bracknell,
Berkshire, UK

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Geochemical and geophysical evidence on the geothermal potential of Caledonian granites in Britain

TECHNIQUES for the extraction of geothermal energy from hot dry granite are being developed with considerable success, principally at Los Alamos, New Mexico^{1–3}. The method involves drilling to depths where the mean temperature exceeds about 160 °C and then using hydraulic fracturing techniques to create an artificial fracture system through which water is circulated and recovered for energy extraction by heat exchangers. At Los Alamos, the required bottom hole temperatures are reached within 3 km of the surface (>50 °C km⁻¹). Although gradients in Britain do not reach this value, a recent economic assessment (J. Garnish, personal communication, based on data in refs 2 and 4) of UK hot rock potential indicates that the Los Alamos technique may become cost effective where the required temperatures occur at <5 km depth; an average minimum thermal gradient of 30–35 °C km⁻¹. The Cornubian granite batholith of Hercynian age and, to a lesser extent the Tertiary granites of northwestern Scotland, have been considered to have potential as hot rock geothermal sources^{4,5}, but less emphasis has been placed on the Caledonian granites of Britain. Recent studies of heat flow in Britain^{6,7}, together with an increasing volume of interpreted geochemical and geophysical data, suggest that certain Caledonian granites of Lower Devonian age may have potential as hot rock sources comparable with that of the Cornubian batholith. Few direct measurements of temperature in Caledonian terrain exist although northern England is

Table 1 Neutron activation analyses for important radioelements in the Cairngorm and Foyers granites

Sample no.	K (%)	U (p.p.m.)	Th (p.p.m.)
Cairngorm			
1	4.85	8.7	29
2	4.89	2.8	14
3	5.96	4.2	24
4	4.58	6.9	20
5	4.07	3.2	15
6	3.08	6.3	37
7	4.37	17.2	25
8	1.92	14.1	16
9	5.24	20.6	33
10	3.59	20.3	32
11	4.91	14.2	37
12	3.84	32.9	39
Foyers			
1	1.48	1.5	3.7
2	1.57	1.7	4.9
3	2.19	0.9	2.2
4	3.19	2.0	12.8
5	<0.80	1.2	6.9
6	2.52	1.5	5.9
7	2.16	1.7	3.5
8	2.75	0.9	3.5
9	2.38	1.3	5.1
10	2.73	0.9	2.7
11	2.81	1.9	4.3
12	3.21	1.3	5.8
Lewisian Inliers			
Mean values	<1.00	<1.0	1.5

U was determined by the delayed neutron method; K and Th were determined by instrumental neutron activation.

known to have high heat flow^{6,7}, and here we review the available data which have a bearing on the geothermal potential of these granites.

The first important feature is radiogenic heat production in the zone of interest. This is because of the known empirical relationship between high surface heat flow and high radioactivity in the crust: $q_0 = q^* + A_0 D$ (where q^* is the probable heat flow contribution from deep sources—the background heat flow— q_0 is the surface heat flow, A_0 is the surface heat productivity and D is a function of the thickness of the radioactive layer^{7,9,10} (see also ref. 8)). The near-surface thermal gradient across a crustal segment is proportional to the measured heat flow (q_0); clearly, this will depend on both radioactivity (A_0) and the value of background heat flow (q^*). For southern Britain, Oxburgh and Richardson⁷ have shown, from limited data, that heat flow and heat productivity are linearly correlated. It follows that in areas where heat flow data are limited it is important to identify zones in which q^* is likely to be most augmented by crustal heat sources. For example, the Cornubian batholith is located in a high heat flow province^{4–6} and is characterised by high contents of the major crustal heat-producing radioelements U, Th and K. Shallow drilling in the Carnmenellis granite to 175 km has indicated gradients between 30 and 35 °C km⁻¹ (ref. 11). The downward continuation of this gradient depends on certain assumptions about the variation of heat production with depth but there are good indications that the area is interesting from a geothermal energy viewpoint, especially where granite is overlain by low conductivity sediments.

In the Caledonian province, much of the region north of the Scottish Midland Valley is apparently underlain by a thick granulite succession¹² which, from geochemical investigations at outcrop^{13–17} and from regional geochemical maps¹⁸, appears to be depleted in heat-producing elements. The cover succession of Moine and Dalradian metasedimentary assemblages contains only low to average crustal abundances of K, U and Th as do the older, pre-Silurian granites, the migmatites and vein complexes,

and the newer forceful granites^{13,14,17,18}. Thus, the northern British Caledonides lack high heat productivity on a regional scale, but this does not exclude the possibility of geothermal hot spots of potential interest which are now tentatively identified.

Regional geochemical maps¹⁸ indicate a high concentration of uranium and potassium over the Cairngorm granite and further studies of the distribution of uranium concentration in British granites¹⁴ have confirmed the relatively high levels in Cairngorm and other Caledonian granites of Lower Devonian age, particularly Etive and Monadhliath from the northern province. To emphasise this point, Table 1 compares neutron activation analyses for K, U and Th in rock samples from Cairngorm, Foyers (a nearby older 'forceful' intrusion) and mean values for some Lewisian inliers. It is clear that Cairngorm has a much higher heat productivity than Foyers and values calculated from Table 1 using appropriate conversion factors¹⁹ are $5.57 \mu\text{W m}^{-3}$ and $0.96 \mu\text{W m}^{-3}$ respectively.

Lower Devonian granites with high heat productivity are a feature of the entire British Caledonides, including northern England which is a recognised high heat flow province^{6,7}: geophysical²² and heat productivity^{13,14,17} data for some of these granites are compared with the Cornubian batholith²⁰ in Table 2. Additional geochemical data for some of these granites indicate that they have features in common with the Cornubian batholith—high levels of K, Rb, U, Th, Li, Be, low K/Rb ratios, low total Sr, low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and chondrite normalised rare earth element (REE) patterns characterised by light RE enrichments with marked negative Eu anomalies^{13,14,17,21}. Both suites of intrusions also have large negative Bouguer gravity anomalies^{21–23} which indicate that they have large volumes and extend to deep crustal levels. Studies of thin sections using the Lexan plastic film technique also indicate that uranium, at least, is located in primary mineral phases such as zircon and apatite^{14,24}. Thus, the high levels of radioelements in these granites at outcrop are unlikely to have resulted from mineralisation. Together with the evidence of shallow drilling in south-west England and Weardale^{11,25} this suggests that the heat productivities in Table 2 may be regarded as representative of larger volumes extending to depth, although this conclusion needs testing by deep drilling and by considering the geochemical evidence for magmatic fractionation. In the absence of heat flow data to the contrary it seems that the essential characteristics of several newer Caledonian granites are comparable with the Cornubian prospect from a geothermal viewpoint.

The factors identified here so far which favour high geothermal gradients within the top few kilometres of crust are: (1) high background heat flow; (2) high heat productivity, normally associated with granites; (3) large intrusive volumes recorded by substantial negative Bouguer gravity anomalies (see also ref. 26).

From Table 2, we deduce that factors (2) and (3) may be as effective in raising thermal gradients over the Cairngorm and Weardale intrusions as over the Cornubian batholith. Unfortunately, Weardale is the only Caledonian intrusion of those listed in Table 2 to have been drilled. Although the thermal gradient within the granite is only 31°C km^{-1} (ref. 27), higher values may be encountered in the coalfield region to the east, within sediments underlain by granite. Such an elevated gradient may be postulated from the much lower thermal conductivity of coal-bearing strata which effectively insulate the underlying region. Another factor favouring high thermal gradients in shallow crust arises: (4) a low conductivity sedimentary cover.

It follows that large volume radioactive granites buried beneath suitable sedimentary sequences provide prime targets for hot rock geothermal exploration. In the British Caledonides such granites may be identified from lows in the Bouguer gravity field since, at outcrop, only this type of granite is associated with high radioactivity.

In conclusion, there are several important geothermal targets in the British Caledonides which might usefully receive further attention (see ref. 28). These include the northern England granites, including postulated buried granites in eastern England (for example, at Market Weighton²⁹) and the region surrounding the Cairngorm and related intrusions. All but the latter lie in a high heat flow province and the Lower Devonian granites of northern Scotland may prove to be relative hot spots in an otherwise barren geothermal province.

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G. C. BROWN

Department of Earth Sciences,
The Open University,
Milton Keynes, UK

JANE PLANT

Institute of Geological Sciences,
154 Clerkenwell Road,
London, UK

M. K. LEE

Institute of Geological Sciences,
5 Princes Gate,
London SW7, UK

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Table 2 Radioelement concentrations, heat productivities and Bouguer gravity anomaly amplitudes for selected Lower Devonian granites from the British Caledonides compared with data for the Hercynian Cornubian granite

Intrusion	K (%)	U (p.p.m.)	Th (p.p.m.)	Heat production* ($\mu\text{W m}^{-3}$)	Amplitude of Bouguer gravity anomaly† (mgal)
Skiddaw	3.86	8.70	18.2	3.91	-19
Weardale	4.29	12.89	12.3	4.63	-37
Shap	4.23	9.68	38.1	5.61	-24
Cairngorm	4.27	12.60	26.8	5.57	-30
Cornubian batholith	4.32‡	11.3‡	19.1‡	4.68	~-50

* Refs 8, 14, 21 and this work.

† Refs 22, 26, 28.

‡ Average from unweathered and unkaolinised samples over entire batholith²³.

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Pliocene eustasy and the onset of sand barrier formation in Gippsland, Victoria

GIPPSLAND possesses a classical development of coastal lakes formed behind a sand barrier system which achieved its present form during the Quaternary. New evidence is assembled here, showing that a transgressive-regressive-transgressive cycle in the late Miocene-Pliocene ended in an earlier phase of barrier formation and lacustrine deposition. Another period of regression in the late Pliocene completed the cycle. Sufficient remnants of the deposits of the Pliocene lake and sand barrier have survived Pleistocene erosion to establish their former existence.

The Gippsland Lakes (lat 38° S. long 148° E.) are a series of brackish and freshwater lakes (Fig. 1), distributed over an area of about 500 km² (refs 1, 2). They overlie a 'continental shelf basin' which has accumulated sediment throughout much of the Tertiary³ and are separated from the Tasman Sea by a sand-barrier system which, in its present form, is undoubtedly Quaternary in age⁴.

This Tertiary sequence contains evidence of a regression in the latter part of the Miocene, first expressed as a facies change from bryozoal to shelly limestone in the upper part of the Bairnsdale Limestone^{5,6} (Fig. 2, phase A) and continuing during the deposition of the overlying glauconitic clays, to culminate in the phosphatic nodule bed (phases B–D). During the regression, the sea retreated from the western (Bairnsdale) area, depositing there only a reduced thickness of Tambo River Formation³. Marine sediments of younger age are known in outcrop only from the Lakes Entrance–Lake Tyers area and in deep wells south and south-west of Bairnsdale⁵. The early Pliocene transgression is expressed by the Jemmys Point Formation, which was deposited in the initially deeper eastern part of the basin as the sea level rose. Deposition at this time did not extend to the Bairnsdale area and did not resume there until the sea level had risen sufficiently. The Jemmys Point Formation is composed of marine silty clay and near its base it contains a molluscan fauna including the tropical bivalve genus *Pinctada*.

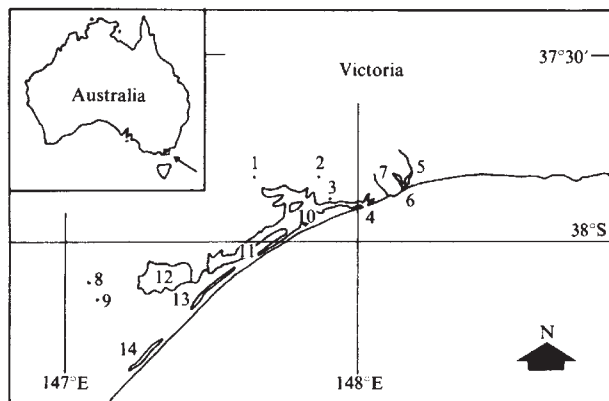


Fig. 1 Locality map of the Gippsland Lakes area. 1, Bairnsdale; 2, Swan Reach; 3, Nungurner; 4, Lakes Entrance; 5, Lake Tyers; 6, Red Bluff; 7, Bunga Creek; 8, Sale; 9, Longford; 10, Lake King; 11, Lake Victoria; 12, Lake Wellington; 13, Lake Reeve; 14, Lake Denison.

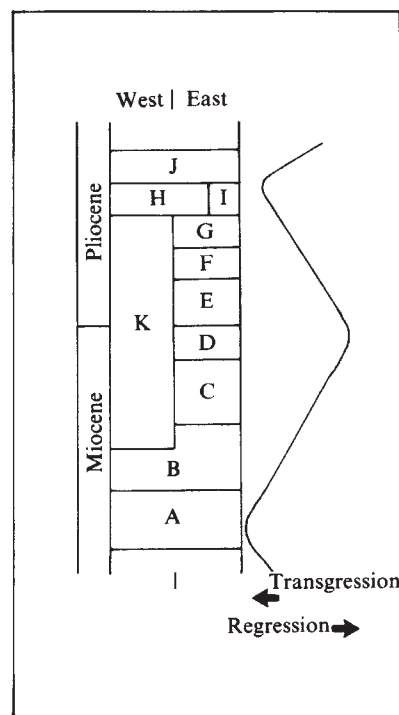


Fig. 2 Stratigraphic column (not to scale) of Miocene–Pliocene formations in Gippsland, correlated with general trends of fluctuations of sea level. Left hand side of column represents western part of area (Bairnsdale district); right hand side represents eastern part (Lakes Entrance–Lake Tyers). A, Bairnsdale Limestone; B, Tambo River Formation; C, Long Point Formation (Carter MS); D, phosphatic nodule bed; E, Jemmys Point Formation; F, Remnant of Pliocene barrier formation within Jemmys Point Formation; G, estuarine beds at top of Jemmys Point Formation; H, Moitun Creek Formation; I, Nyerimalang Formation; J, Haunted Hill Gravel; K, strata missing on unconformity at Bairnsdale.

(the 'pearl shell'), suggesting a warm water environment. It is dated by a lower pelagic foraminiferal zone of *Globorotalia puncticulata*, overlain by a zone of *G. inflata*. In this latter respect, it resembles Pliocene zones in New Zealand⁷.

Just below (1–2 m) the top of the Jemmys Point Formation, a bed of cross-bedded sandstone (Fig. 2, Bed F) is intercalated in the otherwise predominantly argillaceous sediment. In places, it possesses sedimentary structures similar to those described from California in a beach formation resting on an unstable substratum⁸. This bed is traceable along the strike for at least 16 km; its continuity and contrasting lithology are highly significant in a depositional facies not noted for continuity of individual beds. This bed is overlain by a further development of the same argillaceous lithology that comprises the bulk of the Jemmys Point Formation, but never more than 2 m thick. However, it has a more estuarine aspect, as evidenced by the abundance of carbonaceous plant debris in the sediment.

Above this level, near the present coast, the depositional facies changes abruptly to littoral sandstones (up to 10 m in thickness), intertwining with lacustrine sediments (Fig. 3). These latter (Moitun Creek Formation) are traceable intermittently for 60 km westwards from Lake Tyers and remnants can be seen as far away as 90 km to the south-west, near Longford⁹. At some places (for example, Red Bluff and Cross's Landing), sections through the littoral sandstone facies show up to 10 m of relatively coarse sandstone and shell fragments, suggesting barrier remnants; elsewhere (such as at the east side of Lake Tyers and Bunga Creek) finer grained sandstones with occasional marine fossils suggest beach or dune developments and both types of sandstone are seen to intertwine with brown siltstones of lacustrine origin. The extent of the Jemmys Point Formation, landwards of the barrier remnants and overlain by the Moitun

Creek Formation, suggests that the barriers occupied an offshore position at the time of their initial formation.

Noteworthy features of this sequence are that the degrees of overlap suggest that the Miocene transgression extended further inland than the Pliocene transgression, suggesting in turn that the Miocene sea level was relatively higher than that in the Pliocene. During the late Miocene regression, non-marine sediments were not deposited on the emerged sea floor, which was not covered by sediment until the Pliocene transgression, with the unusual event of lacustrine sediments deposited behind the sand barrier being transgressive. However, during the late Pliocene regression, the Haunted Hill Gravel was deposited on the emerged sediment surface composed of Moitun Creek and Nyerimalang Formations. Rapid accumulation of Haunted Hill Gravel is suggested by its poorly sorted character and relatively rapid regression is also indicated.

Recent field work¹⁰ at Lake Tyers has demonstrated a regressive sequence in the mid to late Miocene, from the Bairnsdale Limestone to the phosphatic nodule bed (Figs 2 and 3), a fuller account of which will be given elsewhere. This Miocene regression is widely recognisable¹¹. The resumption of deposition on the Gippsland continental shelf in the early Pliocene, following the regression, is also compatible with world-wide seismic data¹¹ and with the transgressive stratotypes of the Pliocene Stages (Tabianian–Piacenzian) in northern Italy¹², overlain by late Pliocene limestone ('Astian' facies). In the Piacenzian, there is also a lower zone of *Globorotalia puncticulata* and an upper zone of *G. inflata*, though with two intermediate zones¹² which have not yet been recognised in Gippsland. In East Anglia (UK) there are no sediments representing the Miocene transgression, but the phosphatic 'boxstones' of Suffolk occur on an unconformity surface¹³—a stratigraphical position compatible with an interpretation of their formation during a period favourable to world-wide phosphatic deposition in the late Miocene¹⁰. The overlying Pliocene deposits ('Coralline Crag') have been described as submarine banks^{14,15} which in a general sense are not greatly different from sand barriers, suggesting an incidence of coastal sand bar formation in the Pliocene, similar to that in Gippsland. One notes also, transgressive–regressive cycles in the Pliocene of South Australia¹⁶, South Africa¹⁷ and Morocco¹⁸.

The strongly onlapping lacustrine sediments of the Moitun Creek Formation (Figs 2 and 3, Bed H) represent deposition at the time of maximum transgression in the Pliocene, after which another regression occurs. The widespread lacustrine sediments require a barrier to account for their non-marine sedimentary facies and a phase of high sea level to account for their areal extent (see Fig. 3). Non-marine clays, sands and gravels (Haunted Hill Gravel) overlie the sediments of phase H–I and these formed during the late Pliocene regression.

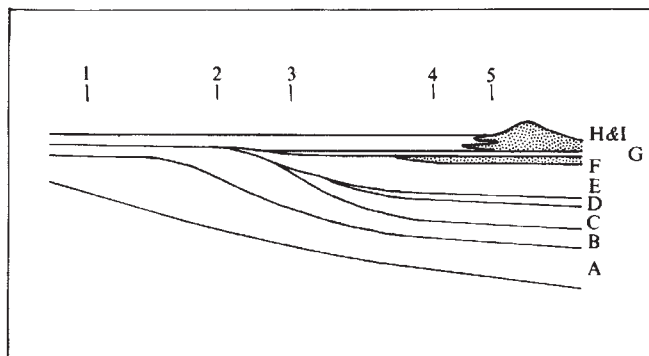


Fig. 3 Reconstruction of the northeastern part of the Gippsland Basin, showing Neogene formations at the end of the Pliocene transgression, before the late Pliocene regression and Pleistocene erosion (not to scale). 1, Bairnsdale; 2, Swan Reach; 3, Nungurner; 4, Lakes Entrance; 5, Lake Tyers. H, Moitun Creek Formation; I, Nyerimalang Formation. Other lettering as in Fig. 2. Stippling indicates sand barrier formations.

During the Pleistocene, coastal erosion destroyed most of the Pliocene barrier and undoubtedly redistributed its sediments during the periods of high sea level and probably also destroyed some of the lacustrine deposits. In the intervening periods of low sea level, river erosion removed large sections of the lake deposits.

It follows from the field evidence that the initial sand barrier formed after a period of rising sea level. Its establishment may have been due to a period of sea level stability but there is no positive evidence either for or against a postulated still stand. It is clear, however, that a further rise of sea level occurred after the initial barrier formation, causing the destruction of the first barrier, whose remnant can be seen (Bed F) but formation of a second barrier (Bed I) and a further rise of sea level allowed the formation of a coastal lake, in which Bed H was deposited.

The presence of Pliocene barrier remnants in Gippsland suggests that, in areas where extensive sand barriers still exist, it would be worthwhile seeking evidence of Pliocene precursors of these barriers, in view of the support they give to the concept of Pliocene eustasy and to the likelihood that they formed at times of still stand during a generally transgressive regime.

A. N. CARTER

*School of Applied Geology,
University of New South Wales,
PO Box 1, Kensington,
New South Wales 2033, Australia*

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Holocene submergence of southern Long Island, New York

RATES of submergence of the northeastern seaboard of the US during the past several thousand years as determined from studies of coastal marsh deposits show considerable variation. The marshes became established on submerging shorelines and have grown upwards and landwards with the continued relative rise in sea level, thereby often forming thick deposits of peat. A series of radiocarbon dates taken at various levels in the marsh can be used to reconstruct the history of submergence. This letter presents a study of the relative change in sea level in southern Long Island, New York, over the past 8,000 yr, and shows that the changing rates of submergence have been an important factor in the initiation and continued development of the area's saltmarshes.

Samples of basal peat from south-central Long Island were dated by the radiocarbon method and a local curve of submergence was constructed for the past 8,000 yr. Three basal-peat samples from marsh areas behind the modern barriers have been dated in the present study. In addition, two previously unpublished dates of organic-rich silty clays (originally back-barrier deposits) from the modern shoreface became available.

Two shallow basal peats from the back-barrier area were collected by hand coring in the saltmarshes fringing the northern margins of western Great South Bay (Fig. 1). Another sample of

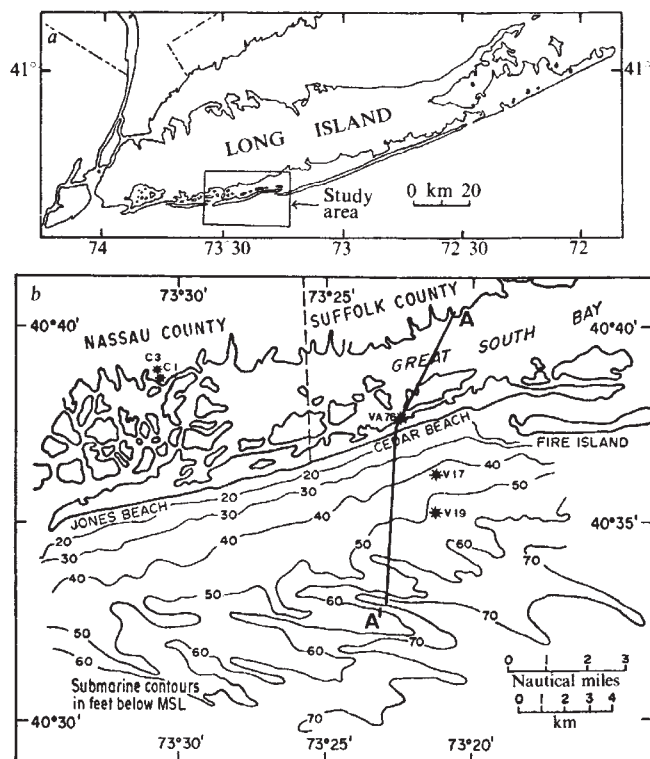


Fig. 1 Location map of the study area showing positions of the borings and cores (*) from which radiocarbon-dated material was taken. Data from the line of borings, A to A', were used to construct the section in Fig. 2. Offshore contours after Williams⁴.

basal peat was collected by vibracoring at a depth of -10 m MSL directly behind and beneath the modern barrier island. Locations of these radiocarbon-dated cores are shown in Fig. 1; the dates are plotted on a schematic cross-section of the Holocene stratigraphic sequence as determined from several hundred boreholes and cores in Fig. 2.

Basal peat in tube core 1 was dated at $1,015 \pm 100$ yr BP (Queens College no QC-315) at approximately -1.1 m MSL. Basal peat in tube core 3 was dated at 300 ± 85 yr BP (QC-316) at approximately -0.3 m MSL. The peat sample taken from vibracore VA-76 at approximately -10.1 m MSL was dated at $5,055 \pm 120$ yr BP (QC-314).

For each of these dates the samples were of salt- to brackish-water peat, directly overlying Upper Wisconsin glacial outwash. Therefore, compaction of the underlying outwash sands and gravels is thought to have been minimal.

Organic silty clays collected by vibracoring on the modern shoreface (Fig. 1) were also dated by the radiocarbon method.

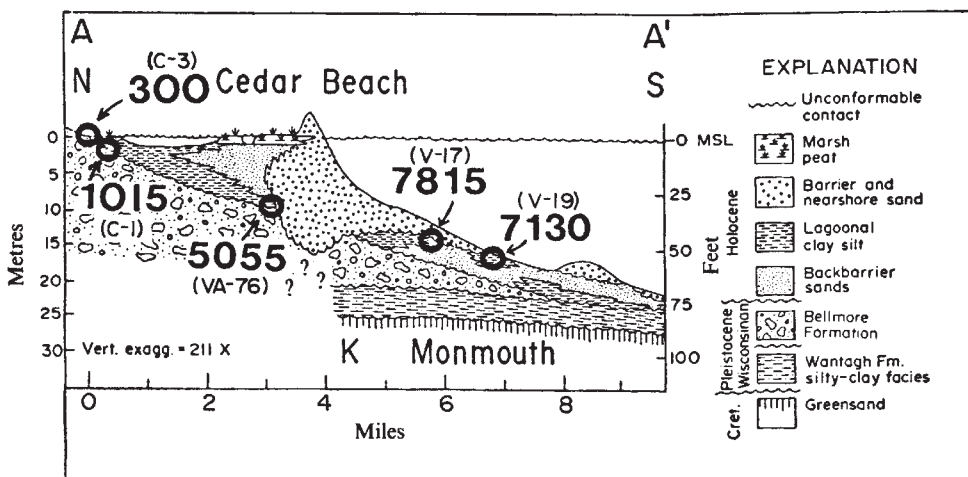


Fig. 2 Radiocarbon-dated samples plotted on schematic, interpretive profile and section A to A' showing the Holocene and Late Pleistocene stratigraphy of south-central Long Island. The positions of samples C-1 ($1,015 \pm 100$ yr BP) and C-3 (300 ± 85 yr BP) are projected from cores to the west of this section.

These former back-barrier sediments were taken from vibracore V-17 at a depth in the core of 1.0 m in 12.6 m of water and from vibracore V-19 at 0.16 m core depth, in 16.4 m of water (Fig. 2). The sediments are lagoonal or saltmarsh deposits now exposed on the shoreface by barrier retreat. These deposits mark the position of a former back-barrier lagoon which existed when the barrier islands were located some 7 km south-east of the present shoreline. The detailed history of the retreat of the barriers of southern Long Island will be discussed elsewhere¹. These back-barrier sediments were probably deposited at or slightly below sea level. They are over-consolidated, which indicates post-depositional compaction. Radiocarbon dating of these silty clays, performed on total organic material in the samples, gave $7,815 \pm 300$ yr BP for sample V-17 (1.0 m) and $7,130 \pm 380$ yr BP for sample V-19 (0.16 m) (dating by Teledyne Isotopes).

These dates from south-central Long Island have been compared with other published dates and curves of Holocene sea level from the Long Island area²⁻⁴, and with the submergence curves which have been constructed for adjacent areas of the Atlantic Coastal Plain⁵⁻⁸ (Fig. 3).

The submergence curve constructed from the data for southern Long Island represents the combined effects of eustatic sea-level rise, local tectonism, tidal variation, water loading and other variables. Therefore, the curve cannot be extrapolated directly to other areas. However, the submergence curve for south-central Long Island can be used in palaeogeographic reconstructions to locate the position of the palaeoshoreline, and as an indicator of changes in the relative rates of submergence during the past $8,000$ yr.

The submergence curve constructed from these data suggests that between $7,000$ and $3,000$ yr BP the Long Island coast was being submerged at a rate of about 25 cm per 100 yr, and that during the past $3,000$ yr, the submergence rate slowed markedly to about 10 cm per 100 yr. Rates of submergence before $7,000$ yr BP are uncertain. Sea-level data presented here show considerable scatter before $7,000$ yr BP (Fig. 3). It has been suggested that, between $9,000$ and $7,500$ yr BP, submergence rates in adjacent coastal areas were as high as 50 cm per 100 yr (refs 9, 10).

The submergence curve for Long Island, therefore, shows a marked reduction in submergence rate at about $3,000$ yr BP, and the possibility of an earlier reduction in the rate of submergence at around $7,000$ yr BP. Studies of relative sea-level rise in other areas of the Atlantic coast have outlined a similar history of changes in the submergence rate^{5,11-13}. For example, the submergence rate for the Delaware coast was approximately 47 cm per 100 yr between $10,000$ and $7,000$ yr BP, slowed to 20 to 30 cm per 100 yr from $7,000$ to $4,000$ yr BP, and has continued at about 8.0 to 12.5 cm per 100 yr for the past $3,000$ to $4,000$ yr (refs 14, 15). Note, however, that the smoothly averaged curves presented here could be reinterpreted in terms of fluctuating relative sea level.

The method of determining relative sea level by point-plotting the ages of basal-peat samples, and then drawing a best-fit curve will average out any fluctuations in the history of submergence. Postulated curves of fluctuating sea level¹⁶ cannot be adequately tested in this manner. Careful stratigraphic analyses are required to test such an hypothesis.

One possible method is to study the stratigraphy of saltmarshes to determine changes in vegetational patterns and shifts in marsh environments that would accompany variations in sea level. No such consistent variations were found in examination of the cores of marsh sediment recovered in the present study.

It has been suggested that changes in the rate of submergence have been an important factor in the development and maintenance of back-barrier saltmarshes^{5,11,17}. The radiocarbon dating of basal peats from southern Long Island sheds light on the mode of formation of the marsh areas. Peat samples from approximate depths of -1.1 and -0.3 m MSL gave radiocarbon ages of $1,015 \pm 100$ and 300 ± 85 yr BP, respectively (Fig. 2). These samples of brackish to saltmarsh peat presumably mark the approximate positions of sea level at those times. The fringing and mid-bay marsh areas of Great South Bay are therefore rather young. Peat has been probed with steel rods to depths of about -2 m MSL which, according to sea-level curves for adjacent areas, would put the inception of these marsh areas at about 2,000 yr BP (Fig. 3).

This conclusion is supported by the report of a basal peat layer at a depth of -7.5 feet (-2.4 m) MSL in Shinnecock Bay, in eastern Long Island, dated at 2,300 yr BP¹⁸. This date indicates that the back-barrier marshes in eastern Long Island were established at least 2,300 yr ago.

It seems that a back-barrier environment, with open-lagoon conditions, existed for some time before the more recent development of the saltmarshes. The colonisation of previously open-lagoon environments by marsh grasses may have been related to a lessening in the submergence rate in the area about 3,000 yr BP. A decrease in the rate of submergence would have allowed sedimentation to build the floor of the lagoon to a level at which the marsh grass *Spartina alterniflora* could grow. As stands of *S. alterniflora* became established and began to trap fine sediment particles, a thin layer of peat would form across former tidal-flat areas. Once the level of the marsh had built to about mean high water, *Spartina patens* could colonise the marsh surface, and marsh building would continue to keep pace with submergence, thus forming the present marshes⁵.

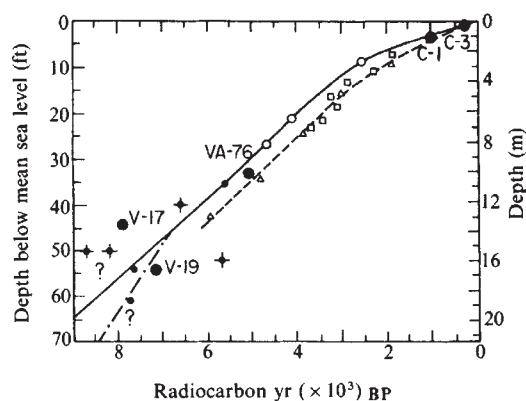


Fig. 3 Submergence curve for southern Long Island during the past 8,000 yr (solid curve). The curve is based on radiocarbon-dated samples reported in this paper (large solid circles) and on previously published dates from northern Long Island^{3,4} (crossed solid circles), southern Long Island^{2,4} (small solid circles) and Iona Island, New York⁷ (open circles). The dashed and dotted curve indicates possible submergence rates of ~50 cm per 100 yr before 7,000 yr BP suggested for adjacent areas^{9,14}. The dashed curve is drawn through points of dated samples from New Jersey⁶ (triangles) and Cape Cod, Massachusetts⁵ (open squares). An assumption of relatively smooth change in sea level has been made in constructing these curves.

In many studies of saltmarshes of the northeastern United States, a relationship has been established between marsh initiation and a marked slowing in the submergence rate between 3,000 to 4,000 yr BP^{5,11,13,17}. It seems likely that the change from open lagoon with intertidal-flat areas to saltmarsh was initiated in western Great South Bay by a decrease in the rate of submergence at about 3,000 yr BP. This slowing of the submergence rate allowed sedimentation to build up the floor of the lagoon by about 2,000 yr BP, to a point at which marsh grasses could colonise former tidal-flat areas along the northern margins of the lagoons and also grow on former tidal-delta deposits. For the past 2,000 yr, upward marsh building has for the most part kept pace with the slow submergence.

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M. R. RAMPINO*

Department of Geological Sciences,
Columbia University,
New York, New York 10027

Received 23 February; accepted 16 May 1979.

* Present address: NASA, Goddard Institute for Space Studies, New York, New York 10025.

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Mössbauer spectral studies of the diagenesis of iron in a sulphide-rich sediment core

ONE stage in sedimentary pyrite formation¹⁻³ is 'FeS' + S → FeS₂, where 'FeS' represents black precursor sulphides such as amorphous iron sulphides and fine-grained mackinawite (Fe_{1+x}S). We report here a Mössbauer spectral study of the formation of pyrite, in sulphide-rich sediments, from the reaction of H₂S with hydrated ferric oxides and with ferrous ions in fine-grained chlorite or in amorphous silicates. Approximately 50% of total Fe deposited is converted to pyrite within a few years, with the remainder being converted over several hundred years. Less than 10% of total Fe is present as precursor sulphides. With suitable constraints in computation, Mössbauer methods show considerable potential for measuring rates of reaction of iron compounds in sediments.

Lake St George is a kettle lake situated 60 km north of Toronto, Ontario. It has a surface area of 10 hectares, a maximum depth of 14 m, and is strongly stratified from May until turnover in late October, with the thermocline at ~9 m. The

surface sediments are rich in organic matter (~25% dry weight) and in sulphide (1.7% total S in the top 6 cm, increasing to ~2.2% below 50 cm, compared with 1.6% Fe). Optical and scanning electron microscopy showed abundant pyrite framboids in all sections of core. X-ray diffraction methods revealed in decreasing order of abundance, quartz, calcite, feldspar, pyrite. Cores were obtained through surface ice in December

1977 using a photoactivated-sphincter lightweight corer⁴. The uniformly grey cores were extruded, sectioned and bottled under nitrogen, to minimise oxidation. The fractions were frozen immediately and freeze-dried 8 h later on return to the laboratory. Mössbauer spectra of the freeze-dried residuals were recorded in January 1978 at University College of Wales, Aberystwyth, and McMaster University, and computer-fitted using Stone's programs⁵. Half widths and peak areas within a quadrupole doublet were constrained to be equal. Low-spin ferrous ions in pyrite and high-spin ferric ions give rise to superimposable doublets^{6,7}, and additional constraints invoked are described below. The Ambrosia pollen horizon occurs at 15 cm, corresponding to colonial settlement in ~1830; this is consistent with current rates of sedimentation of ~1 mm yr⁻¹ calculated from the weight of material collected in sediment traps.

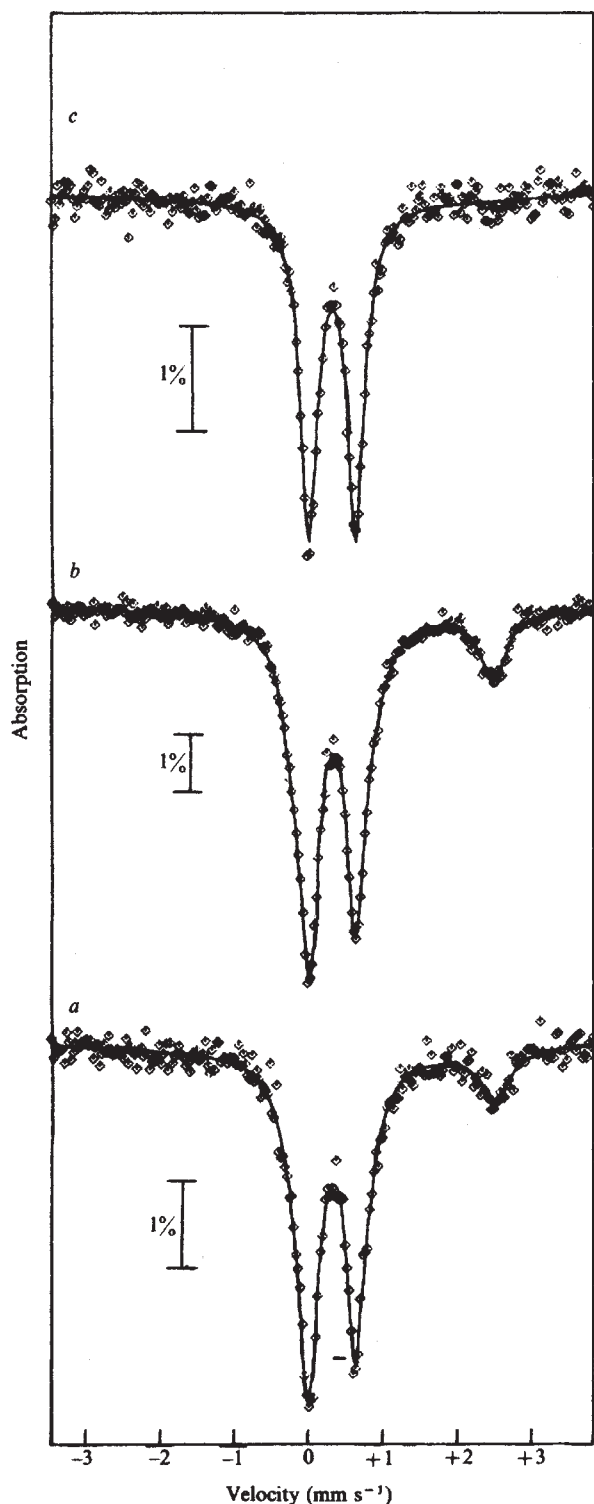


Fig. 1 Room-temperature Mössbauer spectra of freeze-dried Lake St George sediments: a, 0–1 cm; b, 1–2 cm; and c, 86–88 cm fractions. Continuous line represents a computed fit, based on one doublet for the 86–88 cm fraction and on three doublets for the other two. χ^2 values are, respectively, 220 with 239 d.f., 260 with 239 d.f., and 270 with 244 d.f.

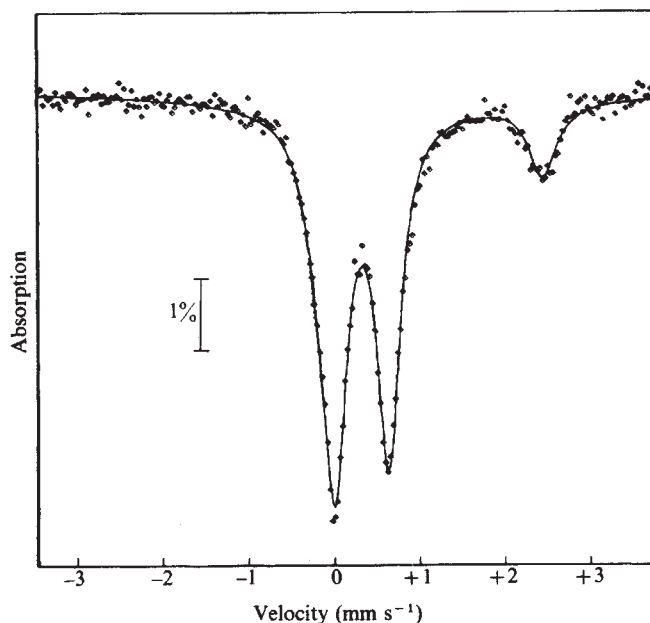


Fig. 2 Room-temperature Mössbauer spectrum of 1–2 cm fractions of Lake St George sediment, showing the two-doublet computed fit as the continuous line. χ^2 value is 339 with 240 d.f.

Selected Mössbauer spectra of sediment residuals are shown in Fig. 1. The disappearance of a 2.6 mm s⁻¹ high-spin ferrous peak in spectra of the deeper fractions is clear evidence for diagenesis. Spectra of 50–52 cm, 62–64 cm and 86–88 cm fractions are virtually identical, and a one-doublet fit yielded values of isomer shift (IS) = 0.31 ± 0.01 mm s⁻¹, quadrupole splitting (QS) = 0.61 ± 0.01 mm s⁻¹, and half width (HW) = 0.285 ± 0.005 mm s⁻¹, which are clearly indicative⁶ of pyrite (Table 1). Similar half widths were measured for powdered pyrite crystals from Tanybwllch Beach, Aberystwyth. Attempts to force-fit the spectra to two doublets by constraining pyrite peak positions and by constraining the peak positions and half widths for amorphous ferric compounds (using values of IS = 0.37 mm s⁻¹, QS = 0.67 mm s⁻¹ and HW = 0.52 mm s⁻¹ measured earlier⁷ for Bay of Quinte, Ontario muds) were either unsuccessful or did not yield significantly lower values of χ^2 . We estimate that >90% of total Fe is in FeS₂; based on 1.6% Fe, the S requirement is ~1.85%. However, 2.2% total S was measured.

Mössbauer spectra of 0–1 cm and 1–2 cm (Fig. 1) and 4–6 cm fractions are virtually identical. A two-doublet fit yielded hyperfine parameters for high-spin Fe²⁺ (the outer doublet) of IS = 1.15 mm s⁻¹, QS = 2.65 mm s⁻¹ and HW = 0.40 mm s⁻¹,

and for the inner doublet corresponding values of 0.32 mm s^{-1} , 0.62 mm s^{-1} and 0.32 mm s^{-1} , all values $\pm 0.01 \text{ mm s}^{-1}$. The former set is reminiscent of octahedrally bonded Fe^{2+} in chlorite⁷⁻⁹, whereas the second set is indicative of pyrite. Compromising the two-doublet scheme are the consistently poor fits on the high-energy limb of the main envelope (Fig. 2) and the significantly larger half widths of the inner doublet (0.32 mm s^{-1}) compared with the 0.285 mm s^{-1} values computed for pyrite in the deeper sections. A three-doublet fit with no additional constraints gave erratic results, so pyrite peak positions and half widths were fixed at values derived from the 50–52 cm, 62–64 cm and 86–88 cm fits. Computed hyperfine parameters and Fe distributions, showing good consistency, are listed in Table 1, together with Fe distribution for 20–22 cm and 44–46 cm fractions. The ferric parameters are in good agreement with those derived for hydrated ferric oxides in muds from other lakes⁷⁻¹⁰.

The results suggest that pyrite forms from the reaction of H_2S with ferrous ions located in fine-grained chlorite or in an amorphous silicate and with ferric ions in mainly amorphous hydrated oxides. In contrast, chlorite-bound ferrous ions in Moira Lake, Ontario, muds (total S $\sim 2\%$, total Fe $\sim 4.5\%$) are not attacked¹⁰ by H_2S . QS values measured for high-spin ferrous ions in Bay of Quinte⁷ and in Lac Phillippe (P.G.M., unpublished work), Quebec, muds are 2.55 mm s^{-1} and 2.50 mm s^{-1} , respectively, suggesting significant differences in structure and/or composition of the host material; the latter value, in particular, seems outside the range for most chlorites⁸. Significant amounts of ferric ion persist for $\sim 200 \text{ yr}$ ($\sim 20 \text{ cm}$ burial) in these reducing sediments, in contrast to conventional views¹¹; they may represent 'aged' (polymerised?) refractory oxides. The mackinawite spectrum¹² is a single peak with a small shift of 0.2 mm s^{-1} ; because of the satisfactory fits obtained with three doublets, it is considered improbable that $>10\%$ of total Fe is in mackinawite. Amorphous Fe–S compounds in the upper few cm are probably oxidised on turnover, but quantitatively similar Fe distributions were obtained in September and early-October samplings. Because such compounds probably contain high-spin ferrous ions which will absorb away from the main envelopes, we estimate that $<10\%$ of total Fe is in amorphous sulphides.

Although the upper few cm of sediment are probably well mixed on turnover, it would seem that $\sim 50\%$ of deposited iron is converted to pyrite within, say, 10 yr, 80% is converted over $\sim 200 \text{ yr}$, and $>90\%$ over $\sim 500 \text{ yr}$. The relative rates are explicable because the concentrations of organic matter and the activity of sulphate-reducing bacteria are greatest in the upper few cm. Framboids were observed in the sediment traps, indicating pyrite formation in the water column during the summer.

Table 1 Hyperfine parameters* and Fe distributions† in Lake St George sediments

Fraction, (cm)	Ferric doublet			Ferrous doublet			Fe distribution (%)		
	IS	QS	HW	IS	QS	HW	FeS_2	Fe^{3+}	Fe^{2+}
0–1‡	0.41	0.73	0.44	1.14	2.71	0.42	57	24	19
1–2‡	0.39	0.75	0.53	1.14	2.66	0.39	53	27	20
4–6‡	0.44	0.67	0.46	1.14	2.64	0.40	58	16	26
20–22§							80	10	10
44–46§							$>85 <15$		
50–52									
62–64							>90		
86–88									

* In mm s^{-1} .

† Assumes equal recoil-free fractions for all compounds.

‡ Pyrite peak positions and half widths fixed in computations.

§ Pyrite and ferric peak positions and half widths fixed in computations.

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P. G. MANNING
J. D. H. WILLIAMS
M. N. CHARLTON

National Water Research Institute,
PO Box 5050,
Burlington, Ontario, Canada L7R 4A6

L. A. ASH

Edward Davies Chemical Laboratories,
University College of Wales,
Aberystwyth, UK

T. BIRCHALL

Chemistry Department,
McMaster University,
Hamilton, Ontario, L8S 4M1

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A new $14 \text{ \AA}$ mineral of the birnessite group in deep-sea micronodules

EXPERIMENTS by Feitknecht and Marti¹ and more recently by Giovanoli *et al.* have shown that oxidation of $\text{Mn}(\text{OH})_2$ in NaOH solution gives rise to a Na-buserite. Drying Na-buserite converts it to Na-birnessite. If a solution of a calcium salt is shaken with Na-buserite the latter is transformed into pseudo-hexagonal Ca-buserite, characterised by a basal spacing of $10 \text{ \AA}$. After drying this spacing is reduced up to $7 \text{ \AA}$ and buserite is converted into Ca-birnessite. We describe here a new natural Ca-bearing member of the birnessite group of unit cell parameters comparable with those of a synthetic compound described² as Na–Mn (II, III) manganate, belonging to the birnessite group. In ASTM (23–1046) this substance is called Na-birnessite.

Dark brown-black nodules about 0.5 mm in diameter have been found in one of the coccolith mud specimens collected in

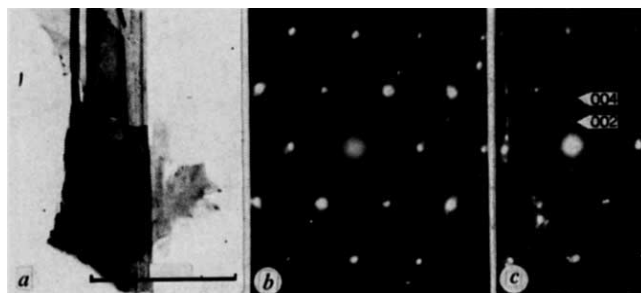


Fig. 1 a, Electron micrograph of $14 \text{ \AA}$ Ca-bearing birnessite. b, SAD-pattern of one platelet corresponding to the plane (001) of this mineral lattice. c, Diffraction pattern of a bent platelet edge. Arrows show the basal reflections 002, 004. Scale bar, $1 \text{ \mu m}$.

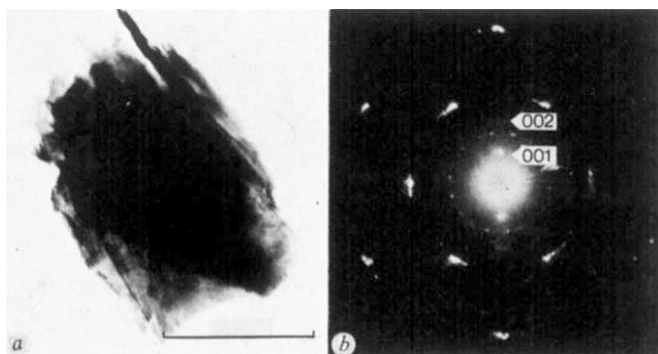


Fig. 2 a, Electron micrograph of 7 Å Ca-bearing birnessite. b, SAD-pattern of a bent edge of this mineral platelet. Arrows show the basal reflections 001, 002. Scale bar, 1 μm.

1977 from the Atlantis fracture zone (Atlantic Ocean) at a depth of 5,520 m (270–280 cm below the sediment–seawater interface), by the 24-th Expedition of RV Academician Kurchatov.

Selected area electron diffraction (SAD) and spectrometer studies of energy dispersion (Kevex) of these nodules revealed two manganese oxide minerals, one of which was named the orthorhombic 14 Å Ca-bearing mineral of birnessite group. The particles of this mineral are elongated platelets (Fig. 1a) ranging in length from fractions of a micron to several microns. When splitting these elongated platelets into still narrower laths, the width range is greater. Many platelets display double or treble intergrowths. The SAD-patterns of single platelets normal to the beam (Fig. 1b) consist of a pseudohexagonal net of closely spaced point reflections. However, an analysis of the diffraction patterns has shown that the hexagonal arrangement is distorted, the extent of distortion varying from particle to particle. SAD patterns of platelets with bent edges and of platelets tilted to the electron beam have been obtained with 00l and hkl reflections respectively (Fig. 1c). From the diffraction pattern data averaged over a number of particles we determined the unit cell parameters as $a_0 = 8.52 \text{ Å}$; $b_0 = 15.1 \text{ Å}$; $c_0 = 14.1 \text{ Å}$. We named this phase orthorhombic 14 Å-birnessite. The established unit cell parameters are almost identical with those of synthetic Na-birnessite of the formula $\text{Na}_4\text{Mn}_{14}\text{O}_{27} \cdot 9\text{H}_2\text{O}$ having: $a_0 = 8.54 \text{ Å}$; $b_0 = 15.39 \text{ Å}$; $c_0 = 14.26 \text{ Å}$. Slight differences in the b_0 and c_0 values can be attributed to a small variation in the degree of vacancy of the octahedral layers, and to a partial dehydration of birnessite in the vacuum conditions of the electron microscope. The mineral of birnessite group with the same unit cell parameter was synthesised in solutions of NaOH as a result of Mn^{2+} oxidation with free oxygen.

The second phase is characterised by slightly elongated laths (Fig. 2a) from fractions of a micron to several microns in diameter. The electron diffraction patterns of their basal planes show a hexagonal net of widely spaced $hk0$ -reflections. Apart from the $hk0$ and $00l$ reflections, hkl space reflections have been observed (Fig. 2b) in many diffraction patterns of the bent edges of particles. The electron diffraction data indicate that the second phase has a hexagonal unit cell with $a_0 = 2.84 \text{ Å}$ and $c_0 = 7.07 \text{ Å}$, both values being in accord with the parameters of common hexagonal 7 Å birnessite.

The Kevex spectrometer analysis of separate single crystal platelets has revealed that the only cations present in all the platelets of natural 14 Å birnessite are manganese and calcium. We can conclude therefore that the structural analogue of synthetic Na–Mn manganate (Na-birnessite) which we found is a 14 Å Ca-bearing birnessite.

The analysis of individual platelets of the 7 Å birnessite mineral with a Kevex spectrometer shows only manganese and calcium. Its diffraction features observed in vacuum conditions, and its chemical composition show that this birnessite is similar to rancieite. That rancieite belongs to the birnessite group (family) has been established by Bardossy and Brindley³.

A few platelets from minerals of the birnessite group were found to contain insignificant amounts of potassium. In one micronodule the crystallites of 7 Å birnessite mineral contain minor amounts of Na in addition to Mn and Ca. Magnesium, which in principle can be detected by the Kevex spectrometer, was not found either in the 14 Å or in the 7 Å birnessite minerals of the studied micronodules.

Hence this study of micronodules from the Atlantic Ocean floor has revealed for the first time a new natural member of the birnessite group.

F. V. CHUKHROV
A. I. GORSHKOV
A. V. SIVTSOV
V. V. BEREZOVSKAYA

*Staromonetny 35,
Institute of Ore Geology,
Moscow, USSR*

Received 9 February; accepted 3 May 1979.

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New fossil finds from the Libyan Upper Neogene site of Sahabi

THE site at Sahabi, Libya, was first investigated in 1934–39¹, and geological, palaeontological and palaeoanthropological research began again in 1977, as part of the International Sahabi Research Project. Further extensive fossiliferous sediments were revealed. A vertical extent of more than 80 m of such sediments are exposed, and on the basis of the mammalian fauna their age is inferred as Late Turolian to Ruscinian, 7–4 Myr BP. We present here a report of Sahabi geology and palaeontology based on results obtained up to March 1979. More than 3,000 invertebrate and vertebrate fossil specimens have been collected, and for several taxonomic groups they fill a gap in the African fossil record.

The fort at Sahabi ('Qasr as-Sahabi') is 90 km south of the coastal town of Agedabia and investigations extend to the north and east (Fig. 1). Sixty-five localities have been established (numbered P1–P65) as stratigraphic profiles or fossil sites.

Table 1 Provisional list of invertebrates from Sahabi

Echinodermata	Bryozoa
<i>Conoclypus</i>	cf. <i>Membranipora</i>
<i>Clypeaster</i>	Arthropoda
Mollusca	Crustacea
Bivalvia	cf. <i>Balanus</i>
<i>Anadara</i>	Ostracoda*
<i>Pecten</i>	Coelenterata
cf. <i>Ostrea</i> *	cf. <i>Montastrea</i>
cf. <i>Crassostrea</i> *	Protozoa
cf. <i>Codakia</i>	Foraminifera
<i>Lucina</i>	Alveolinidae
<i>Glycymeris</i>	Ichnogenera
Mytilidae (cf. <i>Lithophaga</i>)	<i>Ophiomorpha</i>
Gastropoda	<i>Thalassanoides</i>
Conidae	
<i>Strombus</i>	
cf. <i>Mathilda</i>	
cf. <i>Turritella</i>	
cf. <i>Cerithium</i> *	
Trochidae	
Naticidae	

These specimens were identified by A. Ekdale and F. Brown. *Taxa from the Sahabi Formation. Other taxa derive from 'Formation M'.

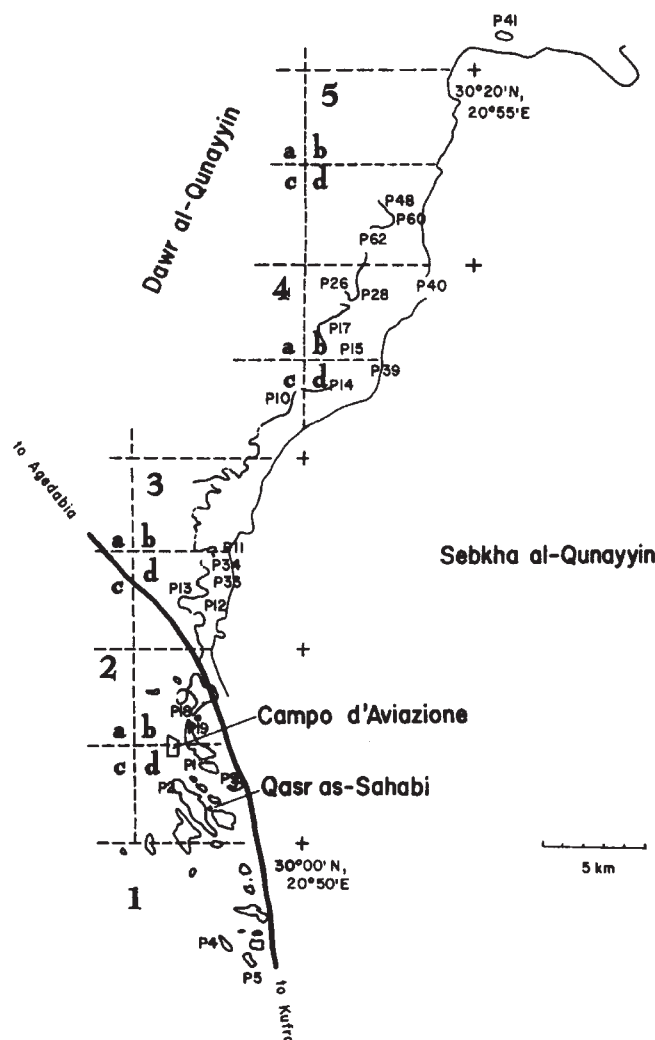


Fig. 1 Map of the major localities at Sahabi. Localities are arranged into sectors which are based on divisions of latitude and longitude. The fort ('Qasr as-Sahabi') and the 1934 Italian airfield are indicated.

De Heinzelin *et al.*² have defined the Sahabi Formation. It lies unconformably on a gypsiferous green clay and sand horizon known as 'Formation P', in turn overlying unconformably Middle Miocene strata ('Formation M'). P may represent deposition during the Messinian Event. Eight lithological members and sub-members (Q,R,S,T,U-1, U-2, V-1 and Z) are distinguished within the Sahabi Formation (Fig. 2). All are fossiliferous with the exception of the highest level, Z. Member Q consists of littoral and lagunal facies followed by shallow marine conditions, as indicated by the deposition of a white dolomite (Member R). Member S records the retreat of the sea by the presence of coastal facies such as lagoons, deltaic channels and freshwater basins. Shallow marine conditions return in Member T, which contains numerous mollusc shells and vertebrates (chelonians, sirenians and cetaceans). The very fossiliferous Member U comprises greenish coarse cross-bedded fluvialite sands as well as lagoonal clays.

Most fossil specimens were found on the surface, although limited excavation and sieving was undertaken. Groups thus added to the Sahabi faunal assemblage include primates, ursids, canids, sirenians, rodents, choerodonts and possibly amebelodonts. Abundant fish, reptile and invertebrate remains are being studied.

All invertebrate remains are of marine origin. Subhorizontal exposures of Formation M north of P39 (Fig. 1) preserve shallow marine, near-shore invertebrate communities, with autochthonous fossils of sea urchins, bivalves and corals. At P40

burrows referred to the ichnogenera *Ophiomorpha* and *Thalassanoides* are preserved. The gastropod *Cerithium* which lives on the bottoms of lagoons is also found in these sediments (personal communication from A. Eckdale and F. Brown). Continuing work on these fossils and on ostracods, diatoms and foraminiferans will contribute to the dating and palaeoecological reconstruction of Sahabi. Table 1 summarises the invertebrate fauna.

The vertebrate fauna is interesting for several reasons. It will provide information about the evolutionary history of several groups whose fossil records are incomplete for the period represented by these sediments. Another advantage of this fauna is that its intermediate position between Africa and Eurasia will make it possible to assess biogeographical connections between the two areas. Palaeoecological studies will be facilitated by subhorizontal exposures which preserve virtually intact fossil surfaces, frequently with *in situ* plant roots, fossil soils and footprints. Fossil wood is abundant and we are looking for pollen in samples of sediments and coprolites.

Abraded and fragmentary bones are abundant in fluvialite and deltaic facies. Rarer but more complete and semi-articulated remains occur in lagoonal and estuarine facies. Transported bone assemblages contain mixed species from different habitats: in locality P16, for example, cercopithecoids, giraffids, *Hipparion*, anthracotheres and sharks (*Carcharodon*) all occur. Table 2 summarises the vertebrate fauna from Sahabi.

The primate remains are the first from pre-Pleistocene strata in Libya. A right half-mandible (1P28A; Fig. 3a) and other postcranial remains are referable to a colobine, *Libypithecus* cf. *markgrafi* Stromer 1913³. Postcranial fossils, including a femur (1P17B) and a proximal ulna (88P36A), show close resemblances to the genus *Macaca* and are referred to cf. *Macaca*. A left clavicle (26P4A) lacking the lateral end has a large sternal articular surface, a large diameter throughout its length, a robust shaft and a strong curvature. The specimen is attributed to Hominoidea, and more precise taxonomic assessment awaits further study.

Sahabi is known for the discovery in 1934 of the type specimen of the proboscidean *Stegotrabelodon lybicus* Petrocchi

Table 2 Provisional list of vertebrates from Sahabi Formation

Cercopithecidae	Giraffidae
<i>Libypithecus</i> cf. <i>markgrafi</i>	? <i>Samotherium</i> sp.
cf. <i>Macaca</i>	Hyaenidae
Hominoidea	<i>Percrocuta</i> sp.
Proboscidea	Felidae
<i>Choerolophodon</i>	<i>Machairodus</i> sp.
<i>Anancus</i>	Ursidae
<i>Stegotrabelodon lybicus</i>	cf. <i>Agriotherium</i>
? <i>Stegodon</i>	Canidae
? <i>Amebelodon</i>	Dugongidae
Rhinocerotidae	<i>Metaxytherium</i> sp.
<i>Brachypotherium</i> cf. <i>lewisi</i>	?Hyracodontidae
Equidae	Rodentia
<i>Hipparion</i> sp. (large)	Ctenodactylidae
<i>Hipparion</i> sp. (small)	spp. as yet unidentified
Anthracotheriidae	Cetacea
<i>Merycopotamus petrocchii</i>	Aves
Hippopotamidae	spp. as yet unidentified
Suidae	Crocodylidae
<i>Nyanzachoerus syrticus</i>	<i>Crocodylus chechchiae</i>
<i>Nyanzachoerus</i> cf. <i>jaegeri</i>	?Tomistomidae
Bovidae	Chelonina
Bovini	cf. <i>Trionyx</i>
<i>Leptobos syrticus</i>	Carcharidae
Boselaphini	<i>Carcharodon</i> sp.
<i>Miotragoceros</i> sp.	Myliobatidae
Hippotragini	<i>Sphyrna</i> sp.
cf. <i>Hippotragus</i> sp.	Centropomidae
Reduncini	<i>Lates</i> sp.
<i>Kobus</i> sp.	

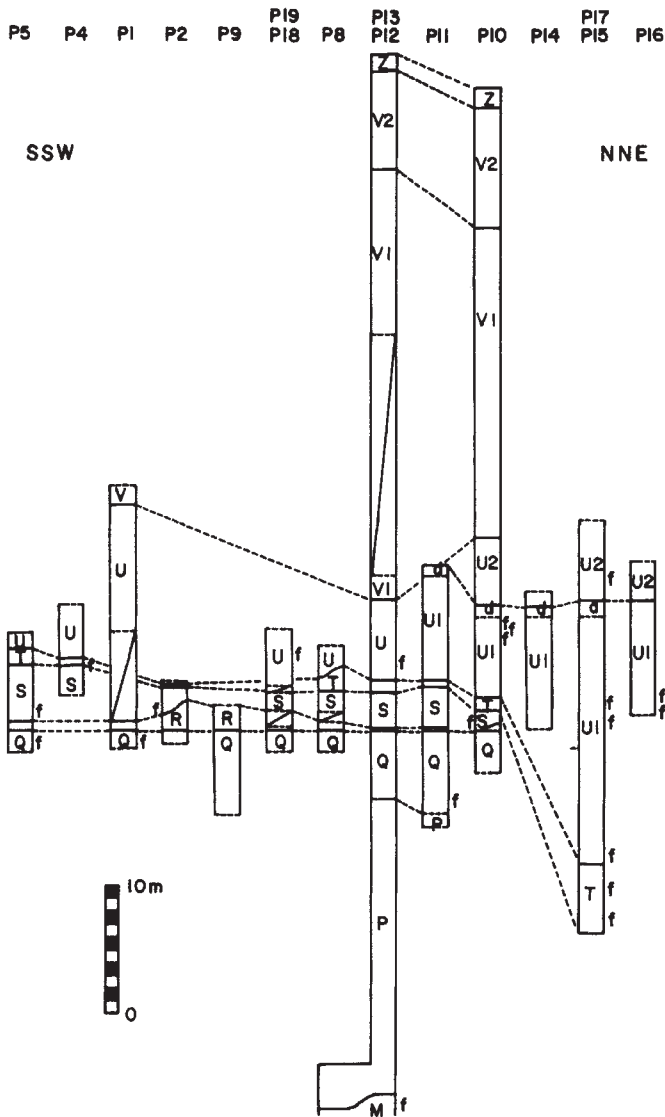


Fig. 2 Stratigraphic sections from selected localities showing 'Formation M; Formation P', and the members of the Sahabi Formation. Major fossiliferous horizons are indicated (after ref. 2). d, Dune; f, fossiliferous horizon.

1941⁴. Diverse proboscideans (Table 2) are now represented by cranial, tusk, molar and postcranial remains. *Stegotetrabelodon* is represented by a complete cranium (1P26A) and fragmentary remains. Upper tusks have almost circular cross-sections and lower tusks have oval cross-sections. The minimum length of a lower tusk is 180 cm. *Anancus* is represented by upper straight tusks with circular cross-sections, and probably also by a right lower third molar with six lophs and a bunodont structure. This specimen resembles that which Petrocchi⁵ ascribed to *Pentalophodon sivalensis*.

Locality P25 yielded the remains of a proboscidean cranium, dentition and upper tusks, the latter being flat in cross-section and with 'rod-cones'^{6,7}. These remains may represent *Amebelodon*. Several bunodont molars with three lophs can be referred provisionally to *Trilophodon* sp. A fragmentary proboscidean molar with only two lophs and no tubercles in the anterior valley could be from a primitive stegodont (*Stegodon*). Another proboscidean molar germ of small size with two lophs has similarities to those of choerodonts and ptychodonts. It resembles molars ascribed to *Choerolophodon* from Anatolia⁷ and is assigned to *Choerolophodon* sp.

Anthracotheres represent one of the most abundant taxa in these sediments. We have provisionally assigned these specimens to *Merycopotamus petrocchii*, the name given by Black⁸ to

specimens found to the north of Sahabi which may or may not be of the same age as those found at Sahabi. The occurrence of hippopotamids of undiagnosed affinities together with anthracotheres is interesting as the latter have been suggested to be ancestral to hippopotamids.

Cooke⁹ has discussed briefly the suids already known from Sahabi and referred them to *Nyanzachoerus syrticus* and *Nyanzachoerus jaegeri*: our discoveries are referable to the same genus. The discovery of an ursid (Fig. 3b) and a canid at locality P28A (U-1) relates Sahabi not only to Eurasian sites but to the South African site of Langebaanweg, dated at approximately 4 Myr, and from which *Agriotherium* and species of Canidae are known¹⁰. The Sahabi forms probably pre-date the South African by at least 1 Myr.

The overall complexion of the Sahabi fauna is African but several taxa indicate Eurasian affinities. These include the boselaphine *Miotragoceros*, the ursid cf. *Agriotherium*, and perhaps the proboscideans *Choerolophodon* and ?*Stegodon* and the cercopitheine cf. *Macaca*.



Fig. 3 a, Lateral view of specimen 1P28A, a right half-mandible ascribed to *Libypithecus* cf. *markgrafi*. b, Occlusal view of specimen 184P28A, an ursid lower molar, tentatively referred to cf. *Agriotherium*.

The nature of the terrestrial fauna indicates marshy to riverine-fringe, wooded environments proximal to the sites of deposition, and more open savanna habitats farther away. Abundant fossils of tree trunks and initial sedimentological findings confirm this reconstruction. Compared with other sites of Turolian to Ruscinian age in Eurasia and Africa, Sahabi presents a high proportion of forest, woodland or water-living forms, including abundant anthracotheres, dugongids, proboscideans, hippopotamids and crocodilids. Taxa more characteristic of open savanna conditions, such as bovids, giraffids, rhinocerotids and equids are less numerous than at contemporary sites¹¹. Cercopithecids and hominoids probably belong to the water-tied or woodland group^{12,13}, which agrees with their absence or rarity at sites with more open habitats lacking surface water, such as Maragheh¹¹.

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NOEL T. BOAZ

Department of Anthropology,
New York University,
New York, New York 10003

A. WAHID GAZIRY
ALI EL-ARNAUTI

Department of Geology,
Gharyounis University,
Benghazi, Libya

Received 12 March; accepted 17 May 1979.

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Insecticide-resistant *Myzus persicae* as an example of evolution by gene duplication

DUPLICATION of structural genes is believed to be a prerequisite for evolution because it allows forbidden mutations of the redundant copy while preserving the advantageous parental gene^{1,2}. Evidence for this phenomenon is derived from studies of proteins with closely related structures which fulfil either slightly or very different roles in metabolism. The first stage of the process, duplication without subsequent mutation, leads to the production of more of a particular gene product. This is especially advantageous during development, when large amounts of ribosomal and transfer RNA are required³. However, so far the only examples of this mechanism causing increased production of specific proteins in response to changing environmental conditions are from studies of bacteria⁴, yeast⁵ and eukaryotic cell cultures⁶. Using insecticide-resistant *Myzus persicae*, we provide evidence here for gene duplication without subsequent mutation, conferring a definite selective advantage to intact higher organisms in adverse environmental conditions.

Needham and R.M.S.⁷ showed a positive correlation between resistance to insecticides of the peach-potato aphid, *M. persicae*, and total carboxylesterase activity measured by the *in vitro* hydrolysis of 1-naphthylacetate. Further work demonstrated that increased esterase activity arises mainly from changes in a single enzyme, E4 (ref. 8). The increased activity is caused by the presence of more E4 rather than a more active mutant enzyme, as shown by measuring the catalytic centre activity of the enzyme from different variants⁸. As E4 hydrolyses insecticidal esters in addition to artificial substrates such as 1-naphthylacetate, it enables resistant aphids having larger amounts of the enzyme to survive otherwise lethal doses by degrading more insecticide⁹.

Genetic studies of increased enzyme production gave ambiguous results^{10,11}, and were hampered by difficulties in breeding the aphids through their sexual cycle. Biochemical studies have now clarified the nature of this phenomenon.

Seven variants of *M. persicae* can be distinguished by esterase assays of either crude aphid homogenates or E4 after electrophoretic separation from other carboxylesterases. Although total esterase assays show a progressive increase in enzyme activity with increasing resistance, quantitative relationships are affected by the hydrolysis of 1-naphthylacetate by esterases other than E4; enzyme staining after electrophoresis only gives an approximate measure of E4 activity. However, the amount of E4 in crude homogenates is readily determined by radioassay with the insecticide paraoxon⁸ (diethyl *p*-nitrophenyl phosphate prepared by oxidation of [ethyl-¹⁴C]parathion (Radiochemical Centre)) as substrate, because only E4 hydrolyses paraoxon⁸.

Weighed groups of adult apterous aphids were homogenised in 0.02 M phosphate buffer, pH 7.0, and incubated at 25 °C with 1.25 µM [ethyl-¹⁴C]paraoxon, a concentration giving V_{max} for the enzyme. The hydrolysis product, diethyl phosphate, was

measured at intervals by liquid scintillation spectrometry of 1-ml samples of the aqueous phase after extracting the unchanged paraoxon into 3 × 1 ml of chloroform. E4 activity (pmol per h per mg aphid) was calculated from these data and the known specific activity (40 mCi mmol⁻¹) of the paraoxon. The results showed that the rate of hydrolysis increased by the same factor between each of the seven variants. Because the catalytic centre activity of E4 for paraoxon is the same in the different variants (0.6 h⁻¹)⁸, the rates give a direct measure of its amount in each variant (Table 1). Statistical analysis¹² using a maximum likelihood computer program confirmed that the amounts in the seven clones form a geometric series with a factor of 1.91 and fiducial limits 1.77 and 2.12. This doubling of E4 throughout the series is best explained by a succession of tandem duplications of the structural gene for E4, with susceptible aphids having one copy, 240N two copies, and so on to G6 with 64 copies of the gene.

Excess production of E4 by *M. persicae*, in common with bacteria⁴, yeast⁵ and eukaryotic cell cultures⁶ showing gene duplication, is unstable in the absence of selection. The two most resistant variants (PirR and G6) spontaneously revert to low esterase activity with concomitant loss of resistance when insecticide selection is removed. This is not observed with the moderately resistant variants (up to and including TIV) in which E4 production is stable.

The three most resistant variants (TIV, PirR and G6) have an A1,3 translocation¹³, unlike the susceptible and the three least resistant variants (with up to eight times as much E4) where karyotype is normal. It has been suggested that this translocation, that is, an exchange between non-homologous chromosomes, or the related dissociation, is responsible for the increased production of E4 by altering interactions between genes on these two chromosomes¹⁴. This is reasonable in view of the location on different chromosomes of some structural genes and their corresponding regulatory genes in *Drosophila*^{15,16}. The clear evidence for gene duplication as the mechanism for increased enzyme production suggests that in this case translocation is more likely to be a mechanism giving rise to tandem duplications. Other mechanisms such as exchanges between homologous chromosomes (unequal crossing over) cannot be excluded, as the karyotype of the less resistant variants is normal. Thus, resistance and the translocation do not show complete linkage as previously suggested¹⁴.

The increased production of E4 clearly confers a selective advantage on *M. persicae* in the presence of insecticides. In Britain, the susceptible variant is now rare, almost certainly as a result of the extensive use of insecticides. It has been almost completely replaced in the field by the MSIG (R₁) variant, with four times as much E4 (ref. 9), and in some areas the even more resistant TIV (R₂) variant is common. So far, the two most resistant variants (with 32 and 64-fold increase in E4) have been widespread only in glasshouses where insecticidal application is much more intensive. These aphids are well adapted to their

Table 1 Concentrations of the insecticide-hydrolysing esterase, E4, in seven aphid variants

Variant	Clone	pmol E4 per mg aphid* ± s.d. (no. of independent determinations)
V1(S)	USIL	0.37 ± 0.20(9)
V2	240N	0.85 ± 0.18(2)
V4	MSIG	1.78 ± 0.75(7)
V8	French R	4.80 ± 0.60(3)
V16†	TIV	6.70 ± 0.70(2)
V32‡	PirR	11.80 ± 1.20(5)
V64‡	G6	24.70 ± 0.20(2)

* Calculated from the rate of paraoxon hydrolysis and the catalytic centre activity of E4 for this substrate.

† Karyotype with A1,3 translocation.

‡ E4 reverts to low activity in the absence of insecticide selection.

environment; they have the ability both to replicate the E4 gene enabling them to survive treatment with insecticides, and to avoid excessive E4 production in the absence of insecticides because the mutational change is unstable¹⁷. This nullifies any possibly adverse effect on fitness through the production of very large amounts of an apparently redundant enzyme.

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A. L. DEVONSHIRE
R. M. SAWICKI

Department of Insecticides and Fungicides,
Rothamsted Experimental Station,
Harpenden, Hertfordshire, UK

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Nerve growth factor alters the fate of embryonic neuroblasts

RECENT work from this laboratory has documented the initial expression of the noradrenergic phenotype in autonomic neuroblasts of the rat embryo *in vivo*. Tyrosine hydroxylase (T-OH) (rate-limiting enzyme in catecholamine biosynthesis)¹, dopamine- β -hydroxylase (DBH) (which converts dopamine to noradrenaline) and the catecholamine (CA) transmitters appear simultaneously in sympathetic ganglion primordia at 11.5 days of gestation^{2,3}. In addition, these noradrenergic characters transiently appear in a population of presumptive neuroblasts within the primitive gut mesenchyme^{2,3}. The noradrenergic gut neuroblasts appear at 11.5 days and increase in numbers over the ensuing 24 hours. Subsequently, however, numbers decrease progressively so that by 14.5 days of gestation virtually no cells exhibiting noradrenergic traits are detectable^{2,3}. Elucidation of the fate of these neuroblasts may help define mechanisms influencing neuronal phenotypic expression and/or the factors which govern selective neuronal death during ontogeny. In this report we show that treatment with the trophic protein macromolecule, nerve growth factor (NGF), increases the number of catecholamine (CA)-fluorescent cells and delays the ontogenetic disappearance of CA from the gut.

NGF is present in various sympathetic target organs, and is necessary for normal survival and growth of sympathetic neurones *in vivo* and *in vitro* (see refs 4 and 5 for reviews). Conversely, treatment of neonates with antiserum to NGF results in widespread destruction of sympathetic neurones throughout the animal^{6,7}. As NGF has a critical regulatory role in sympathetic ontogeny, it was logical to define the effect of this agent on the noradrenergic neuroblasts of the embryonic rat gut *in vivo*.

At 11.5 days of gestation embryos were injected with NGF and the gut was examined for CA-containing neuroblasts. The β subunit of NGF was purified as before⁸. Pregnant Sprague-

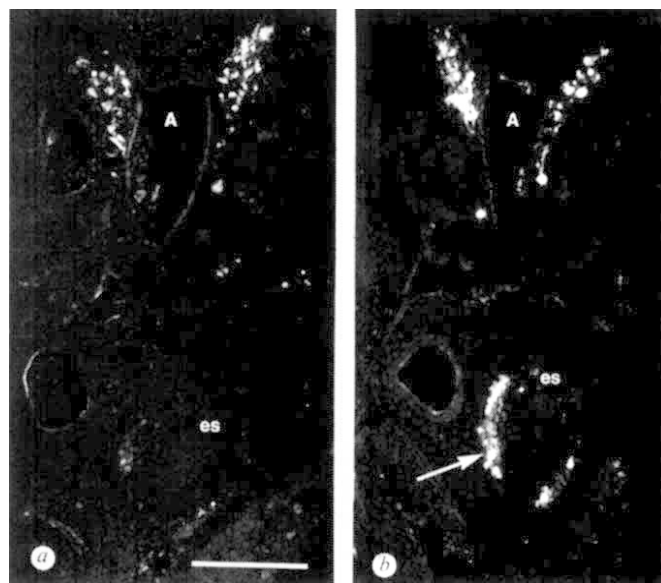


Fig. 1 Early effects of NGF treatment on CA-containing neuroblasts of the gut. Embryos were injected with either vehicle (a) or 50 BU of β -NGF in 5 μ l (b) at 11.5 d of gestation; 2 d later the embryos were processed for the histochemical demonstration of CA. a, Mid-thoracic transverse section of control (vehicle-treated) embryo. CA-histofluorescence is evident in sympathetic primordia located on either side of the aorta (A). Note a small number of faintly fluorescent cells in the gut mesenchyme, adjacent to the oesophageal epithelium (es). b, NGF-treated embryo. Transverse section at same anatomical level as in a. The number and fluorescence intensity of CA-containing cells in the gut mesenchyme have increased dramatically (arrow). The neuroblasts are aggregated in ganglion-like clusters surrounding the oesophageal epithelium (es). The ganglion primordia adjacent to the aorta (A) are not significantly altered. Scale bar, 200 μ m.

Dawley rats were anaesthetised⁹, and trans-uterine injections of embryos were performed by opening the mother's peritoneum and exposing and trans-illuminating the uterus. The visualised embryos were injected using a 33-gauge needle with either β -NGF or control buffer. Animals were killed at daily intervals thereafter, from 12.5 to 15.5 days, and CA was detected by formaldehyde-induced fluorescence¹⁰.

Treatment with NGF was associated with a dramatic increase in the number and fluorescence intensity of noradrenergic neuroblasts in the gut. These effects were most striking 2 days after injection, at 13.5 days of gestation (Fig. 1). In the oesophagus, at the level of the gastro-oesophageal junction, neuroblast numbers were markedly increased by NGF, and cells aggregated to form ganglion-like clusters (Fig. 1b). In contrast, far fewer CA-containing cells were detected in vehicle-treated controls, and fluorescence intensity was diminished (Fig. 1a). NGF induced similar increases in fluorescent neuroblast numbers and in cellular aggregation in the proximal small intestine. Although effects decreased progressively with time after injection, changes were still evident in 15.5-day embryos, the oldest specimens examined. NGF treatment caused persistence of scattered fluorescent neuroblasts in the gut of 15.5-day embryos, whereas control guts were devoid of fluorescent cells (Fig. 2). Preliminary observations suggest that the gut neuroblasts are sensitive to NGF only during a restricted period of ontogeny; treatment at 10.5 or 13.5 days of gestation had little effect.

In addition to increasing fluorescent cell numbers in the gut, NGF treatment increased the number of apparently ectopic neuroblasts in unusual locations. Fluorescent neuroblasts were observed in the liver parenchyma, the heart primordium, the somatopleural mesenchyme of the body wall and in some instances, in the limb bud mesenchyme (Fig. 3) after NGF administration.

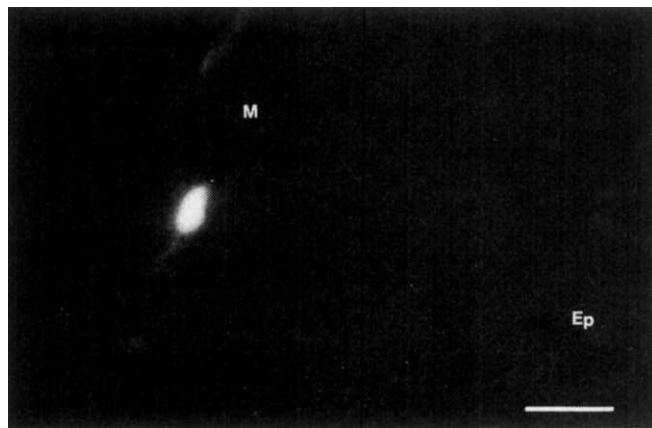


Fig. 2 Late effects of NGF treatment. Embryos were injected with vehicle or β -NGF at 11.5 d of gestation as in Fig. 1, but were examined 4 d later, at 15.5 d of gestation. An isolated fluorescent cell with a fluorescent cytoplasmic process was observed in the developing circular muscle layer of the intestine (M). Such isolated, CA-containing cells were present throughout the intestines of NGF-treated embryos, whereas the guts of controls were completely devoid of fluorescent cells. Ep denotes epithelium of the gut. Scale bar, 20 μ m.

In contrast to effects elsewhere, NGF had little apparent effect on neuroblasts in the sympathetic ganglion primordia (Fig. 1). Treatment did not alter neuroblast numbers and had inconsistent, minimal effects on fluorescence intensity in the primitive ganglia. However, embryonic ganglia are responsive to NGF¹¹. Consequently, our observations suggest that the exogenous dose of NGF may have been too small, compared with local endogenous concentrations, to elicit changes.

NGF may have increased the number of noradrenergic gut neuroblasts by (1) increasing the number of undifferentiated precursors that acquire noradrenergic traits; (2) causing persistence of noradrenergic traits in neuroblasts normally destined to acquire and then lose these characters; (3) causing mitosis of noradrenergic neuroblasts; (4) lengthening survival times of noradrenergic neuroblasts; (5) increasing the CA content of noradrenergic cells to the level necessary for visualisation. Alternatives (1) and (2) imply that NGF regulates phenotypic expression directly, whereas (3)–(5) suggest that NGF regulates cell number, and/or CA content, but not phenotypic expression

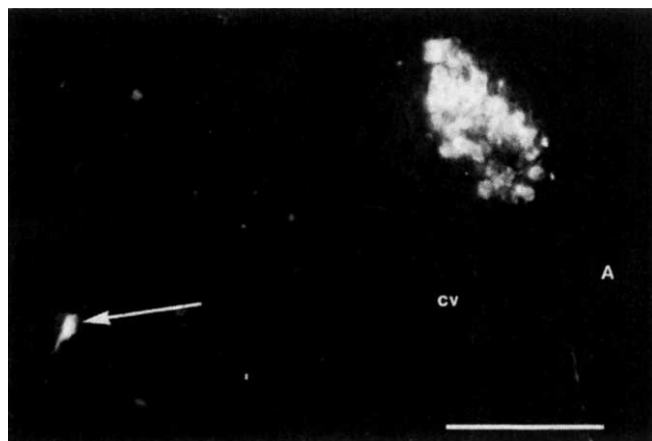


Fig. 3 Appearance of ectopic cells after NGF treatment. High thoracic transverse section of a 13.5-d embryo. An isolated fluorescent cell (arrow) is observed in the mesenchyme of the proximal portion of the limb bud. The sympathetic ganglion primordium, located between the aorta (A) and the cardinal vein (cv), is a great distance from the ectopic cell. NGF treatment increased the number of ectopic CA-containing cells in a variety of unusual locations. In contrast, ectopic fluorescent neuroblasts were very rarely encountered in controls. Scale bar, 100 μ m.

per se. It is certainly well documented that NGF enhances sympathetic neurone survival during development^{12,13}. However, the protein does not apparently alter transmitter phenotypic expression. Rather, NGF increases acetylcholine synthesis in cholinergic neurones, and noradrenaline synthesis in noradrenergic neurones^{14–17}. It remains to be determined whether NGF influences gut neuroblasts in the rat embryo in an analogous manner. Regardless of the mechanism involved, our observations suggest that NGF can increase the number of noradrenergic neuroblasts which can be visualised in the gut and ectopic locations, and cause persistence of such cells. It is possible, consequently, that local concentrations of endogenous NGF normally determine which neuroblasts will survive and/or continue to express noradrenergic characters.

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JOHN A. KESSLER
PHILIPPE COCHARD
IRA B. BLACK

Laboratory of Developmental Neurology, Cornell University Medical College, Department of Neurology, 1300 York Avenue, New York, New York 10021

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Appearance of H-W (H-Y) antigen in the gonads of oestradiol sex-reversed male chicken embryos

THERE is evidence that H-Y antigen has a major role in testis differentiation in mammals^{1–3}. Presumably, this antigen is the essential factor for the development of the undifferentiated gonadal anlage into a testis. Antiserum to H-Y antigen, raised in highly inbred strains of mice and rats, exhibits cross-reactivity with cells derived from non-mammalian vertebrates like birds, amphibians and fish^{4,5}. In these animals, the cross-reaction is strong in the heterogametic sex. This means that in birds with the ZZ/ZW mechanism of sex determination the female types as positive, as also occurs in the toad *Xenopus laevis*; on the other hand, in the frog genus *Rana* and the cyprinodont fish *Lebistes* and *Xiphophorus*, which have the XX/XY mechanism of sex determination, the male types as positive. This correlation with the heterogametic sex favours the assumption that the cross-reacting antigen 'H-Y' or 'H-W' in non-mammalian vertebrates

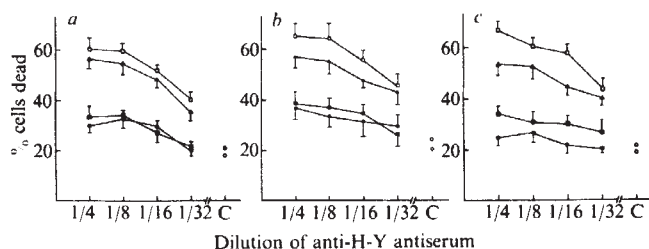


Fig. 1 Cytotoxicity of anti-H-Y antiserum in the presence of rabbit complement after absorption by 25 mg wet weight of pooled embryonic chick gonads. Mean and standard deviation of six determinations each are shown. $\square$, Dead cells at different dilutions of anti-H-Y antiserum; $\blacktriangle$, dead cells at different antiserum dilutions after absorption by left control testes; $\blacksquare$, dead cells at different antiserum dilutions after absorption by oestradiol sex-reversed left ovotestes; $\circ$, dead cells at different antiserum dilutions after absorption by left control ovaries; $\circ$, complement control. *a*, 13-d-old embryonic gonads; *b*, 14-d-old embryonic gonads; *c*, 16-d-old embryonic gonads.

has a similar function to that occurring in mammals for the differentiation of the heterogametic gonad, that is, the ovary in ZW, and the testis in XY animals. In contrast to placental mammals, in the other vertebrates it is possible to influence gonadal differentiation by sex steroid hormones (for review, see ref. 6). In the embryonic male (ZZ) bird, application of oestrogens leads to the development of an ovotestis, in amphibians and fish, treatment with steroid hormone of the opposite sex causes complete sex reversal. If H-Y (H-W) is the differentiation antigen for the heterogametic gonad, and if on the other hand sex reversal can be achieved by the steroid hormone of the opposite sex, the question arises whether the hormone acts by way of the H-Y (H-W) antigen. Therefore, we have converted embryonic male chickens using oestrogen to develop an ovotestis, and then tested them for the presence of cross-reactivity with rat anti-H-Y antiserum. We report here that, indeed, the normally antigen-negative testis becomes antigen-positive after hormonal treatment. Thus, the gene for this antigen must be present in the genome of both sexes.

Embryos of white Leghorn chickens were used for the experiments. Eggs were injected with 100 μ g oestradiol benzoate on day 4 of incubation as described, for example, by Akram and Weniger⁷, and killed on days 13 or 16. Male (ZZ) converted gonads were definitely distinguished from female ones by morphological criteria. The feminised testis has the flattened form of the left ovary, but it is much smaller and its inner and outer borders have indentations, in contrast to the smooth aspect of the left ovary. The feminised right testis is longer and thinner than the right female gonad. The müllerian ducts of the feminised embryo resemble those of the female embryo, except that the left one lacks a shell gland. All these criteria taken together make the diagnosis of the genetic sex quite definite. In only three out of more than 100 cases was a decision difficult; these three embryos were discarded. Thus, gonads regarded as feminised testes were without doubt of ZZ constitution. In an additional experiment, eggs of the Ross Brown breed, which shows sex-linked differences in feather colouration, were used. Thus, this breed offers an independent criterion for sexual assignment. The plumage is brownish in females and white in males. These characters are already clearly distinct in 12-d-old embryos, and allow the genetic sex of the oestrogen-treated embryos to be determined. Sex ratios obtained according to the above criteria were:

16 d, I (Leghorn):	19♂ 24♀
16 d, II (Leghorn):	6♂ 7♀
13 d, I (Leghorn):	12♂ 15♀
13 d, II (Leghorn):	16♂ 12♀
14 d (Ross Brown):	30♂ 26♀

Gonads were collected in liquid nitrogen immediately after preparation and weighing, and stored at -80°C . Untreated left

testes and ovaries of embryos of the same ages served as controls. Only left gonads were used throughout the experiments, as in the female the right gonad is rudimentary. H-Y antiserum was raised in female isogenic Lewis rats by several injections of male spleen cells as described earlier⁸. Cytotoxic testing for the detection of H-W antigen was carried out essentially according to Scheid *et al.*⁹: 20 μ l 1/4 diluted antiserum was absorbed with 25 mg wet weight of pooled gonads derived from the respective type of embryo and tested for cytotoxicity on male rat epidermal cells. For each test 10 μ l absorbed antiserum were used, the remainder being stored at -80°C and tested for confirmation later on. Five experiments were carried out, two experiments each for yielding 13- and 16-d-old embryonic gonads, respectively, and another one for 14-d-old gonads of the Ross Brown breed. For each experiment about 80 eggs were used, about 50 of which were treated with oestrogens.

Figure 1*a*, *b* and *c* demonstrates the results of conversion experiments finished on days 13, 14 and 16, respectively. Converted gonads of 13- and 14-d-old embryos absorb almost as much antiserum as do normal female gonads, whereas those of 16-d-old embryos absorb a little less than do control ovaries. No elevated titres of H-W antigen were found in oestrogen-treated ovaries.

These findings clearly indicate that, after treatment with oestrogens, H-Y (H-W) antigen becomes expressed in the male gonad. This suggests that, analogous to the well studied situation in mammals, in birds the presence of H-W antigen is responsible for the formation of an ovary; if this is true, oestrogen-induced sex reversal, which is well known (for review, see ref. 6), is only an indirect effect, mediated by H-W antigen. In mammals, attempts to induce H-Y antigen by testosterone have been reviewed by Erickson¹⁰. Evidently, however, the primary differentiation of the gonadal anlage is independent of sex steroids in mammals (for review, see ref. 6).

It may be significant that hormone-induced sex reversal is not complete in the chick. In general, an ovotestis develops which gradually regresses towards hatching. Although, we were not able to study H-W antigen during the entire course of embryonic development, in the gonads of 13- and 14-d-old embryos, the antigen titre was clearly stronger than in those at 16 d. Therefore, there does seem to be a correlation between morphogenetic changes and the H-W titre in the gonad. Apparently, the 'induction' of H-W antigen production by oestrogen does not function by means of a switch-on mechanism resulting in the continuous production of the antigen (there must be some interaction with a repressing or degrading factor).

The results obtained in this study also have implications for the sex specificity of the H-W structural gene. Induction of H-W expression in the male bird presupposes the existence of the respective gene in the 2A, ZZ genome. Therefore, W-linkage of the H-W structural gene is excluded; this gene must be shared by both sexes. In mammals, however, the H-Y structural gene is assumed by many authors to be Y-linked¹¹ and consequently absent in the female. This question, however, cannot be considered as settled¹². In addition, because in lower vertebrates sex reversal can be achieved by appropriate treatment, the gene responsible for the primary differentiation of the heterogametic gonad must be present in both sexes. From an evolutionary point of view, we believe therefore that in mammals also, this gene is not sex specific, and only its state of activity determines whether the undifferentiated gonadal anlage becomes a testis or an ovary.

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U. MÜLLER
MARIA T. ZENZES
U. WOLF

*Institut für Humangenetik
und Anthropologie der Universität,
Albertsstraße 11, 7800 Freiburg, FRG*

W. ENGEL

Institut für Humangenetik der Universität,
Nikolausbergerweg 5a,
3400 Göttingen, FRG

J.-P. WENIGER

Laboratoire de Zoologie
et d'Embryologie Expérimentale,
Université Louis Pasteur,
12, Rue de l'Université,
67000 Strasbourg, France

Received 2 January; accepted 1 June 1979.

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Preimplantation lethality of monosomy for mouse chromosome 19

It remains a mystery why autosomal trisomy is found more frequently than autosomal monosomy in man. Several surveys of spontaneous abortions have revealed these chromosomal abnormalities in proportions of 26% (ref. 1) and 0.1% (refs 1, 2), respectively, while a prospective study of 47,000 newborn babies revealed no full monosomy and only five cases in which parts of chromosomes were missing³. Similarly, in mouse embryos from translocation stocks bred to produce aneuploid progeny, high frequencies of trisomy but no monosomy are found after 12–13 days^{4,5}. This is hard to understand because

Table 1 Frequency of aneuploid preimplantation mouse embryos

Chromosome	Day	No. of embryos		
ICR females		Total	Monosomic	Trisomic
1	3	19	3(16%)	3(16%)
	4 all	54	5(9%)	9(17%)
	in vivo	13	1(8%)	2(15%)
	in vitro	41	4(10%)	7(17%)
12	3	72	10(14%)	11(15%)
	4 in vitro	38	5(13%)	5(13%)
17	3	23	2(9%)	5(22%)
	4 in vivo	48	4(8%)	6(13%)
19	3	116	21(18%)	23(20%)
	4 all	194	3(2%)	46(24%)
	in vivo	65	0(0%)	15(23%)
	in vitro	129	3(2%)	31(24%)
C57BL/6J females		Total	Monosomic	Trisomic
19	3	55	9(16%)	11(20%)
	4 all	84	1(1%)	16(19%)
	in vivo	16	0(0%)	4(25%)
	in vitro	68	1(1%)	12(18%)

Aneuploid embryos were generated by mating males doubly heterozygous for two Robertsonian translocations^{5,6} to superovulated ICR-Swiss or C57BL/6J females. The following male stocks were used: Rb1Bnr/Rb10Bnr for aneuploidy of chromosome 1, Rb5Bnr/Rb9Bnr for chromosome 12, Rb11Rma/Rb11em for chromosome 17 and Rb 163H/Rb1Ct for chromosome 19. Preimplantation embryos were obtained on the third (day 3) or fourth (day 4 *in vivo*) day after observation of the plug (day 0). Day 4 *in vitro* embryos were obtained by culturing⁹ day 3 embryos for 24 h. The embryos were karyotyped as before¹⁷.

Table 2 Development of aneuploid day 3 embryos from ICR × Rb163H/Rb1Ct matings

Karyotypes	Total no.	Stage of development reached			
		8–16 cells	Late morula	Early blastocyst	Blastocyst
Normal	18		9	8	1
Trisomy 19	6	1	2	3	
Monosomy 19	6			3	2
Undeterminable	10		7	1	2

Day 3 embryos were scored for development and then karyotyped as previously described¹⁷.

most autosomal aneuploidy is assumed to result from meiotic nondisjunction, so that for every gamete carrying two copies of a particular chromosome there should be a complementary gamete carrying none. However, the deficiency of monosomic embryos could be because nullisomic gametes are less able than disomic gametes to participate in fertilisation, or because the resulting monosomic embryos are less viable early in gestation. We report here the investigation of these possibilities in a mouse system which is known to produce a high frequency of nondisjunction. Equal numbers of monosomic and trisomic progeny were found early in gestation, but while some monosomies were compatible with survival to implantation, monosomy for the smallest mouse autosome, chromosome 19, was lethal at the early to mid-blastocyst stage. This lethality did not seem to be the result of the expression of recessive lethal genes.

Mouse embryos aneuploid for chromosomes 1, 12, 17 and 19 were generated by the double metacentric technique^{5,6}. Because of indications^{5,7} that most monosomic embryos do not survive much beyond implantation, we used preimplantation embryos for our initial studies. The frequencies of aneuploid embryos were analysed on days 3 (late morula/early blastocyst) and 4 (late blastocyst) of development (Table 1). In each case, the doubly heterozygous parent was the father. For each of chromosomes 1, 12 and 19, the frequencies of monosomy and trisomy were virtually the same on day 3 and varied from 14 to 20%. Therefore, for these three mouse chromosomes we conclude that there is no selection against nullisomic sperm, that equal numbers of monosomic and trisomic gametes are produced at fertilisation and that the survival of monosomics to day 3 is relatively normal.

The situation was different on day 4. While the frequencies of trisomies 1, 12 and 19 and monosomy 12 each remained about the same as on day 3, the frequency of monosomy 1 had decreased somewhat and that of monosomy 19 had decreased considerably, from 18 to 2%. This loss of embryos lacking chromosome 19 occurred both *in vivo* and *in vitro*. When day 3 embryos were cultured overnight, a significant number of them died and the frequency of monosomy declined to 2% (Table 1). However, no viable monosomics were found among embryos obtained directly on day 4 (Table 1), and the inviability of monosomy 19 cannot therefore be attributed to some increased sensitivity to *in vitro* culture conditions. There were too few chromosome 17 aneuploids to draw firm conclusions about their overall viability. Nevertheless, the data suggest that at least some monosomics are viable to the late blastocyst stage.

The day 3 embryos listed in Table 1 were a mixture of late morulae and blastocysts. Because the transition between morula and blastocyst is particularly sensitive to various agents, including α -amanitin⁸ and bromodeoxyuridine⁹, and is the time when development stops in embryos homozygous for the t^{12} mutation¹⁰, we investigated whether it was affected by monosomy 19. Day 3 embryos were scored visually and then karyotyped (Table 2). Both normal and trisomic embryos consisted of approximately equal numbers of late morulae and early blastocysts. Similarly, half of the identified monosomics had also blastulated, while it was not clear whether the rest had blastulated. Thus

blastulation itself does not appear to be the vulnerable step in monosomy 19, but the period immediately after does.

One possible explanation for the very early lethality of monosomy 19 is that a recessive allele, able to cause death before implantation, becomes uncovered in the monosomic state. Although the only chromosome 19 present in the embryos is that derived from the normal but outbred ICR Swiss parent, this does not seem likely. A very high frequency of such a lethal allele would be necessary to explain the death of all the embryos monosomic for chromosome 19. This would, in turn, result in the death of many normal embryos at the blastocyst stage, but there was no evidence for significant lethality among the hundreds of day 3 ICR blastocysts surviving in culture to day 4. Nevertheless, to obtain further evidence against the recessive lethal hypothesis, double metacentric-bearing males were mated with inbred C57BL/6J females and the progeny were scored as before (Table 1). Again, approximately equal numbers of monosomics and trisomics were observed on day 3, but as before, virtually no monosomic embryos survived to day 4. Therefore, we conclude that the lethality of monosomy 19 is an inherent property of the aneuploid state itself and does not depend on the hemizygous expression of recessive lethal alleles.

Data complementary to ours have been published by Dyban and Baranov¹¹. After 3 days of development, no embryos monosomic for chromosome 5 or 17 were found. The proportions of embryos monosomic and trisomic for chromosomes 15 or 19 were the same, but both types of monosomics were retarded in development and had low cell numbers.

Chromosome 19, which comprises only 2.7% of the haploid set by length, is the smallest of the mouse chromosomes, and trisomy 19 is the trisomy associated with the greatest survival and the only one compatible with viability beyond term (Table 3)¹². Survival of trisomies 1, 12 and 17 is much more limited¹³. By contrast, of the four monosomies studied, monosomy 19 is clearly the most lethal. Therefore, there does not seem to be any reciprocity between the viability of the monosomies and of the corresponding trisomies. Likewise, from the data in Table 3 and the more extensive data obtained by others from human trisomic embryos^{14,15}, there is no obvious relationship between the overall size of a chromosome and the survival of embryos which are aneuploid for it¹⁶. Although it has been suggested that there is an inverse correlation between the viability of specific human trisomies and the amount of active chromatin, as judged from Giemsa negative or R-band positive regions in the chromosomes involved¹⁴, such a relationship does not exist for the mouse aneuploids discussed here (Table 3). We conclude therefore that the ultimate effect of each specific aneuploidy, and particularly of the monosomies, is as much, if not more, a function of the particular loci affected than of the total number of genes or amount of chromatin present on the chromosomes.

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CHARLES J. EPSTEIN
BRUCE TRAVIS

Department of Pediatrics,
Department of Biochemistry and Biophysics and
Howard Hughes Medical Institute Laboratory,
University of California, San Francisco,
California 94143

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Selective suppression of IgE antibody responsiveness by maternal influence

IMMUNOGLOBULIN E antibodies are produced in two circumstances: by all normal animals in infections with helminth parasites^{1,2}, and by certain individuals against common environmental substances³. In these latter 'atopic' individuals, immediate hypersensitivity reactions mediated by IgE are responsible for various disorders, which in man include hay fever, allergic asthma and allergies to foods and drugs. It is known that the antibody response of the young animal may be reduced or enhanced by maternal immunisation⁴⁻⁹. We have examined the antibody response of rats immunised with egg albumin (EA) and show here that both these effects can occur together: suppression of the IgE antibody response may occur against a background of enhanced total antibody responsiveness in the offspring of immunised mothers.

Our study was originally stimulated by observations that animals including man can occasionally be born hypersensitive or with specific IgE antibody in the cord blood¹⁰⁻¹³. As IgE does not cross the placenta¹⁴ its presence in the neonate indicates fetal synthesis following *in utero* stimulation with antigen. We reasoned that suppression of IgE response could also result from such an antigenic stimulus and might in fact be a more frequent occurrence. Consequently, we fed or injected rats during pregnancy and lactation with different quantities of EA given without adjuvant. In this way, the mother would act merely as a vehicle for conveying antigen to the fetus without herself producing an IgE response, as this requires that the inducing dose of antigen be administered together with an adjuvant¹⁵. We found that such treatment of the mothers did not have any influence on the subsequent IgE responsiveness of the offspring.

These experiments, however, led to others in which we examined the effect of immunising rats with antigen and adjuvant long enough before a pregnancy to allow the development of an immune response. Prospective mothers were immunised with either 10 mg or 10 µg EA together with *Bordetella pertussis* as adjuvant 1 month before mating (Fig. 1). These doses of antigen had previously been shown to generate two different types of response: namely, specific IgE response in the rats

Table 3 Relationship of survival of aneuploid mouse embryos to chromosome length and Giemsa light banding content

Chromosome	Length (% of haploid set)		Survival (d)	
	Total	Giemsa light bands	Monosomics	Trisomics
1	7.2(2.7)	3.1	≥4	14-15
12	4.6(1.8)	2.2	≥4	14-17
17	3.7(1.4)	1.3	≥4	10-12
19	2.7(1.0)	1.0	3.5	≥19

Chromosome lengths are as reported by Nesbitt and Francke¹⁸, and the lengths of the Giemsa light band regions are calculated from Fig. 1 of the same report. The figures in parentheses are the ratios of chromosome lengths to the length of chromosome 19. The survivals of the trisomic embryos are as reported by Gropp *et al.*¹³ and White *et al.*¹², and of the monosomics as reported here.

immunised with the lower dose and superimposed specific IgE suppression in those immunised with the higher dose^{15,16}.

The results show that the offspring of the mothers immunised with either dose had a greatly depressed IgE response to EA. Feeding EA during pregnancy did not influence the subsequent IgE responsiveness of the progeny. Similarly, injection of the mothers with adjuvant alone was without effect.

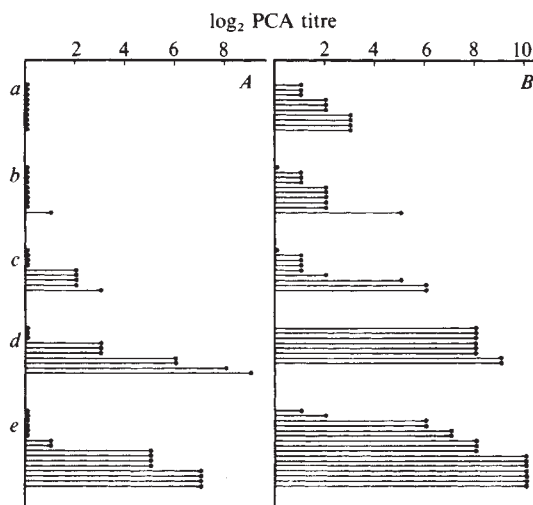


Fig. 1 The effect of preimmunisation of female rats or of feeding antigen during pregnancy on the specific IgE antibody responsiveness of the offspring. Outbred female Hooded Lister rats (Animal Suppliers) weighing 150–200 g were immunised 1 month before mating with the indicated amounts of egg albumin (EA) (Sigma grade V) injected intraperitoneally in 0.15 M NaCl together with a suspension of 10^{10} heat-killed *B. pertussis* as adjuvant. In addition the rats of groups *b*, *c* and *d* received EA in the drinking water at $10 \mu\text{g ml}^{-1}$ for 10 d starting 9 d after mating. Animals of a group immunised with $10 \mu\text{g}$ EA without additional antigen feeding did not become pregnant. The female offspring (two to three litters per group), which were born within 5 d of each other, were immunised with $10 \mu\text{g}$ EA + *B. pertussis* 6 weeks after the birth of the last litter and were challenged with $1 \mu\text{g}$ EA 35 d later. Blood for antibody assay was collected by severing the tip of the tail. IgE antibodies were estimated by the passive cutaneous anaphylaxis technique as previously described¹⁰. The figure shows the primary and booster IgE responses of the individual animals 12 d after immunisation (A) and 4 d after challenge (B). The times of bleeding were determined by previous observations on the kinetics of these responses in Hooded Lister rats¹⁵. Treatment of mothers: *a*, Immunised with 10 mg EA 1 month before mating; *b*, Immunised with 10 mg EA 1 month before mating and EA in the drinking water during pregnancy; *c*, Immunised with $10 \mu\text{g}$ EA 1 month before mating and EA in the drinking water during pregnancy; *d*, EA in the drinking water during pregnancy; *e*, No treatment.

The suppression of IgE responsiveness was antigen specific. This was demonstrated by inoculating the offspring of EA-immunised or normal mothers with both EA and keyhole limpet haemocyanin (KLH). The IgE response to EA but not to KLH was depressed in the offspring of the EA-immunised mothers.

To determine whether the suppressive effect was transferred transplacentally or in the milk, the offspring of EA-immunised and untreated mothers were exchanged on the day of birth. The IgE response of these rats following immunisation at 5–6 weeks of age was compared with that of rats reared by their natural mothers. As shown in Fig. 2 the IgE response to EA was suppressed in the offspring of normal rats if suckled by previously immunised mothers whereas the IgE response was normal in the offspring of immunised mothers fostered to non-immunised mothers. This indicated that the factors responsible for suppression were transferred in the milk. When further litters were cross-fostered at 1 or 2 weeks of age, specific suppressive factors from the immunised mothers were found to be transferred during the entire period of lactation. Thus, if the offspring of normal mothers were suckled by immunised mothers for even 1 week (from days 15–21 of lactation) a significant degree of suppression was transferred.

Assessment of diminished antibody response in the progeny of immune mothers—by measuring total antibody or numbers of antibody-producing cells, or by observing functional effects such

Table 1 Comparison of EA IgG and total antibody response with EA IgE levels in the offspring of normal (A) and immunised (B) mothers

	IgE (geometric mean PCA titre)		IgG (mean c.p.m. $\times 10^{-3}$ ($\pm$ s.d.))		Total antigen- binding capacity (mean $\log_{10}$ μg EA bound per ml serum $\pm$ s.d.)	
	A	B	A	B	A	B
Day 20 after immunisation	9.18	All negative	1.92 (pool)	2.93 (pool)	0.97 (0.15)	1.35 (0.19)
Day 4 after challenge	1,175	All negative	4.45 (2.12)	4.71 (1.43)	1.39 (0.20)	1.47 (0.18)
Day 21 after challenge	278	All negative	3.39 (1.05)	3.72 (2.62)	1.71 (0.36)	1.49 (0.20)

IgG response to EA was measured by a paper radioallergosorbent test in which paper disks to which EA had been coupled were reacted first with serum and then with ^{125}I -labelled anti-rat IgG. The method is fully described elsewhere¹⁹. The antigen-binding capacity was measured by the Farr technique²⁰ as modified by Mitchison^{21,22}. Iodination of EA was carried out with the labelled ester Bolton and Hunter reagent²³ (*N*-succinimidyl-3-(4-hydroxy, 5- ^{125}I -iodophenyl) propionate; Radiochemical Centre using 5 mg EA and 1 mCi of the labelled reagent. The measurements shown were made on five serum samples from each of groups *a* and *b* rats in the experiment shown in Fig. 2. The difference between groups A and B on day 20 after immunisation in the antigen-binding assay is significant ($P < 0.01$).

as vaccination failure—has hitherto been made without reference to possible differential action on antibody class. To determine whether the suppression we had observed in the offspring of immunised mothers was selective for IgE or merely a facet of a generally suppressed response, we measured the IgG response and total antigen-combining capacity for EA in the sera of normal and IgE-suppressed offspring. The results in Table 1 show that higher levels of IgG and total antibody were produced in the primary response to antigen by the offspring of immunised mothers. Examination of the sera of progeny before immunisation showed that antibodies to EA (excluding IgE) were passively transferred from the immunised mother, but declined to scarcely detectable levels by 6 weeks. The additive effect of residual maternal antibody could therefore not explain the higher level of primary response seen in the progeny of immunised mothers.

Reduced antibody production in the offspring of immunised mothers has usually been attributable to maternal antibody

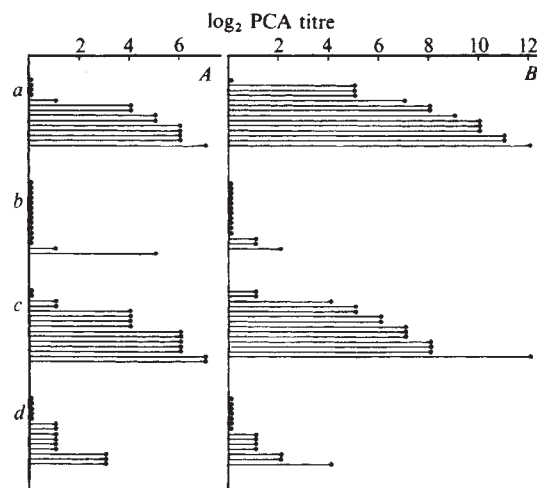


Fig. 2 Effect of cross-fostering on the transfer of the IgE suppressive effect. The immunised mothers were injected with 1 mg EA and 10^{10} *B. pertussis* 1 month before mating; control mothers were untreated. At birth the offspring were either left with their own mothers or were exchanged so that those born of immunised mothers were suckled by non-immunised mothers and vice versa. These exchanges were carried out as soon as possible and not more than 1–2 h after birth. Each of the groups *a*–*d* consisted of the female rats of four litters. These rats were immunised at 5–6 weeks with $10 \mu\text{g}$ EA and *B. pertussis* and were challenged 1 month later with $1 \mu\text{g}$ EA alone. The results shown are the primary and booster EA IgE antibody responses of individual rats. 20 d after immunisation (A) and 4 d after challenge (B). Groups: *a*, offspring of normal mothers; *b*, offspring of immunised mothers; *c*, offspring of immunised mothers fostered to normal mothers; *d*, offspring of normal mothers fostered to immunised mothers.

passively transferred transplacentally and/or in the milk according to species^{17,18}, although tolerance induction following encounter with antigen by the same routes has also been suggested^{5,6}. We do not know the mechanism of suppression of IgE responsiveness nor whether it is related to the enhanced total antibody response. However, because a maternal immune response is necessary the phenomenon must be mediated by immune factors.

Maternal immunity transferred to the offspring for protection against infection may also interfere with the development of active immunity. The latter can be seen as a side effect, a price to be paid to ensure the survival of the neonate. However, transfer of IgE suppression from mother to young can be regarded as an objective. For, whatever the biological role of IgE, a response to substances such as pollens or foods apparently leads only to a deleterious hypersensitivity. To control such aberrant production, elaborate and sensitive immunoregulatory mechanisms have been evolved. The existence of an allergy-protective device involving specific IgE suppression by maternal influence is an additional possibility which must now be considered.

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ELLEN JARRETT
ELIZABETH HALL

Department of Veterinary Parasitology,
University of Glasgow, Veterinary Hospital,
Bearsden Road, Bearsden, Glasgow, UK

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T cells primed by influenza virion internal components can cooperate in the antibody response to haemagglutinin

THE fact that nude mice produce significantly lower titres of antibody to influenza haemagglutinin than do normal mice implies that this response is T-cell dependent^{1,2}. Further evidence of cooperation between T and B cells has been provided by the immune response of mice to purified haemagglutinin³: a greater production of strain-specific, but not of cross-reactive, antibody was observed in the presence of haemagglutinin-primed T cells. We report here that the antibody response to haemagglutinin can also be enhanced by T cells primed by the internal components of the influenza virus. This may provide an explanation for previous findings^{4–6} that infection with one

Table 1 X31 HAI antibody response of mice after antigen priming

Priming antigen	Boosting antigen	log ₂ serum HAI titre ± s.e.m.	
		Day 4	Day 10
—	X31 Virus	≤1.58*	2.38 ± 0.49
X31 Spikeless particles	X31 Virus	4.98 ± 0.93	5.38 ± 1.07
—	X31 Monomer HA	≤1.58	2.33 ± 0.75
X31 Spikeless particles	X31 Monomer HA	≤1.58	≤1.58
X31 Spikeless particles	X31 Spikeless particles	≤1.58	≤1.58

Groups of five CBA/T6 mice were primed by i.p. injection of 100 µg *B. pertussis* plus 10 µg antigen as stated. After 28 d the mice were boosted by i.p. injection of 10 µg antigen and bled 4 and 10 d later.

* Limit of assay: no antibody detectable.

influenza subtype may influence the subsequent response to another which does not cross-react serologically.

Treatment of the X31 strain of A/Hong Kong/1/68 influenza virus (ref. 7) with the protease bromelain removes the haemagglutinin and neuraminidase subunits from the surface of the virion and leaves the particles smooth-surfaced⁸. Spikeless particles prepared in this way were injected intraperitoneally (i.p.) into 12-week-old CBA/T6 female mice together with *Bordetella pertussis* (Wellcome). When subsequently injected i.p. with purified X31 virus, mice primed with spikeless particles gave a much higher and earlier haemagglutination inhibition (HAI) antibody response than did controls that had been injected with *B. pertussis* alone (Table 1). In contrast, the response to bromelain-extracted monomer haemagglutinin⁸ was not enhanced by priming with spikeless particles. There was also no HAI antibody response to a second injection of spikeless particles. Similar results were obtained from preliminary experiments using deoxycholate-treated virus as the priming antigen. This preparation lacks both envelope lipid and surface glycoproteins but contains all the internal proteins and RNA of the virus⁹. These results suggest that the priming effect is unlikely to be mediated by otherwise undetectable traces of haemagglutinin remaining in the X31 spikeless particle preparation.

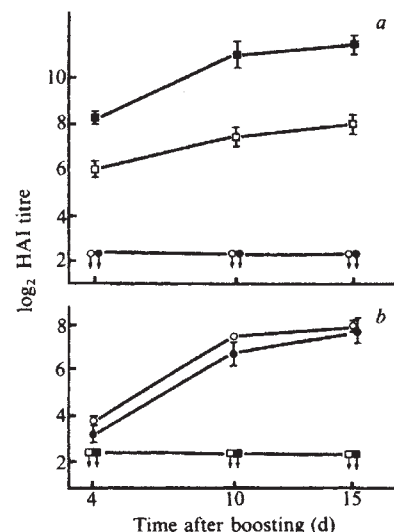


Fig. 1 a, A/PR/8/34 HAI antibody response; b, B/Victoria/70 HAI antibody response. Geometric mean serum antibody titres in groups of five mice. Bars indicate standard errors. □, Normal mice immunised with 10 µg A/PR/8/34; ■, mice primed with 10 µg X31 spikeless particles and boosted with 10 µg A/PR/8/34; ○, normal mice immunised with 10 µg B/Victoria/70; ●, mice primed with 10 µg X31 spikeless particles and boosted with 10 µg B/Victoria/70.

Mice primed with X31 spikeless particles and then injected with A/Puerto Rico/8/34 virus gave an enhanced HAI antibody response to this subtype (Fig. 1a), even though the H₃ haemagglutinin of X31 is antigenically distinct from the H₃ haemagglutinin of A/PR/8/34. The HAI antibody response of mice to B/Victoria/70 was not influenced by priming with X31 spikeless particles (Fig. 1b), indicating that the priming is type specific.

The cell type responsible for the priming effect was investigated using a spleen cell transfer technique (Table 2). Recipient mice given 10⁸ unfractionated spleen cells from primed donors were able to mount an enhanced response after i.p. injection of X31 virus. Ig-negative spleen cells separated by the rosette method, which removes more than 97% of Ig-bearing cells as detected by autoradiography¹⁰, were also effective; 2 × 10⁷ Ig-negative spleen cells were capable of substituting fully for 10⁸ unfractionated cells. Smaller quantities were progressively less effective, but still produced a measurable improvement in antibody response. We have not found it necessary to irradiate the recipient mice in our adoptive transfer experiments. This may be because relatively few primed cells are required. It may also reflect a more general property of the virus, as successful adoptive transfer of influenza immunity using normal recipients has previously been reported^{11,12}.

Our findings strongly suggest that cell cooperation analogous to that seen in the carrier-hapten phenomenon¹³⁻¹⁵ occurs in the antibody response to influenza haemagglutinin. Thus, T cells recognising some internal component of the virion are capable of cooperation with B cells in the production of HAI antibody. Work in progress indicates that of the internal components at least the matrix protein is recognised by cooperating cells (Table 3). The mechanism by which associative recognition of the haemagglutinin and internal proteins can occur is unclear. The haemagglutinin spikes are present on the virus surface and do not seem to penetrate the lipid envelope, whereas the matrix protein, the outermost of the internal components, is contained within the lipid envelope and is thought not to penetrate the bilayer to the virion surface¹⁶. The two proteins are therefore connected and also separated by the lipid bilayer. Thus, for recognition and cell cooperation to occur, either the matrix protein is more accessible than described above or some processing step may be necessary to present both proteins in association on the surface of a macrophage or other cell.

In this latter case, the mechanism of help is similar to that of the associative recognition of intrinsic cell surface proteins described by Lake and Mitchison¹⁷. In their terminology this type of effect could be described as intermolecular, intrastructural help, as both helper and principal determinants are carried by different proteins. Our data then provide an example of cell-surface associative recognition in which both antigens are of viral origin.

It has been reported that the HAI antibody response of ferrets, hamsters and mice to inactivated influenza vaccines can

Table 3 Ability of purified matrix protein to prime helper cells

Priming antigen	log ₂ serum HAI antibody titre ± s.e.m.	
	Day 4	Day 11
—	≤1.58*	4.58 ± 0.77
X31 Spikeless particles	4.30 ± 1.12	6.38 ± 0.37
Matrix protein	3.98 ± 0.68	7.18 ± 0.51

Groups of five CBA/T6 mice were primed by i.p. injection of 100 µg *B. pertussis* plus 10 µg antigen as stated. After 28 d the mice were boosted by i.p. injection of 10 µg purified X31 virus and bled 4 and 11 d later. Matrix protein was derived from A₂/Jap/305/57 × A₀/Bel/42 (H₂N₁), prepared by the method of Laver¹⁸. SDS-polyacrylamide gel electrophoresis gave a single distinct protein band.

* Limit of assay: no antibody detectable.

be enhanced by prior heterotypic infection^{4,5} and even that infection can provide a partial heterotypic immunity⁶. Our results may help to explain these otherwise puzzling phenomena.

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S. M. RUSSELL

Department of Virology R & D,

F. Y. LIEW

Department of Experimental Immunobiology,
The Wellcome Research Laboratories,
Beckenham, Kent, UK

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Table 2 Adoptive transfer of the priming effect by Ig-negative cells

Donor cells	log ₂ serum HAI antibody titre ± s.e.m.	
	Day 4	Day 10
—	≤1.58*	5.78 ± 0.58
1 × 10 ⁸ Normal spleen cells	≤1.58	4.78 ± 0.37
Primed spleen cells:		
1 × 10 ⁸ Unfractionated	3.98 ± 0.24	5.98 ± 0.24
2 × 10 ⁵ Ig-negative	3.58 ± 0.00	5.98 ± 0.51
2 × 10 ⁶ Ig-negative	3.78 ± 0.20	5.78 ± 0.37
2 × 10 ⁷ Ig-negative	4.78 ± 0.20	6.33 ± 0.25

Groups of five normal CBA/T6 female mice were injected with spleen cells from donors primed 28 d earlier with 10 µg X31 spikeless particles. Spleen cells were fractionated by the rosette method¹⁰. Recipients were boosted with 10 µg X31 virus and bled 4 and 10 d later.

* Limit of assay: no antibody detectable.

Use of cyclosporin A in allogeneic bone marrow transplantation in the rat

ALLOGENEIC bone marrow transplantation (BMT) has become the therapy of choice for the treatment of severe aplastic anaemia and severe combined immunodeficiency diseases and is considered beneficial for the treatment and possible cure of malignant diseases, especially lymphohaematopoietic malignancies¹. Several serious complications, however, prevent the full realisation of the therapeutic potential of this procedure. To accept an allogeneic marrow graft, patients (even those treated for aplastic anaemia) have to be conditioned with cytoreductive agents like cyclophosphamide (Cy) or total body irradiation (TBI), agents that have relatively low therapeutic indices for immunosuppression and marked toxicities. Furthermore, patients, even if matched with their respective donors at the major histocompatibility complex (MHC), have after engraftment successfully, a high incidence of potentially fatal graft-versus-host disease (GvHD). Third, patients, who successfully engraft have a severe immunodeficiency for several months after transplantation that apparently contributes to potentially fatal

Table 1 Use of Cs A as an immunosuppressant to establish engraftment in BU-treated rats

Group	Treatment at day			Days after grafting											
	-8	-1	0	Mort.	7 Chim.	GvHD	Mort.	14 Chim.	GvHD	Mort.	21 Chim.	GvHD	Mort.	28 Chim.	GvHD
1		BU	—	0/8	—	—	8/8	—	—	—	—	—	—	—	—
2		BU	Lewis	0/8	—	—	0/8	—	—	0/8	—	—	1/8	—	—
3		BU	ACI	0/8	—	—	8/8	—	—	—	—	—	—	—	—
4	RARTS	BU	ACI	1/8	0/7	0/7	2/8	6/6	4/6	4/8	4/4	4/4	7/8	1/1	1/1
5		BU+Cy	ACI	0/8	0/8	0/8	0/8	8/8	3/8	3/8	5/5	5/5	5/8	3/3	3/3
6	Cs A	BU	ACI	0/8	0/8	0/8	0/8	NT	0/8	0/8	8/8	0/8	1/8	7/7	0/7

Groups of Lewis rats were treated with busulphan 30 mg per kg i.p. alone (groups 1, 2 and 3) or together with cyclophosphamide 150 mg per kg i.p. (group 5), a short course of rabbit anti-rat-thymocyte serum i.p. (RARTS, 1.5 ml daily from day -8 to day -2) (group 4) or Cs A (25 mg per kg given subcutaneously daily from day -8 to day +18) (group 6). One day after busulphan administration 60×10^6 nucleated marrow cells were given intravenously. Cumulative mortality, fraction of animals proven to be chimaeras and fraction of animals with clinical signs of GvHD are recorded. Chimaerism was established by typing the peripheral lymphocytes with strain-specific cytotoxic antisera. NT, Not tested.

opportunistic infections in the post-grafting period². Attempts to overcome these complications have had only limited success. Cyclosporin A (Cs A), an undecapeptide of fungal origin³, has been reported to have very profound antilymphocytic activities with very low degrees of myelotoxicity⁴. It has been shown to suppress a variety of humoral and cellular immune phenomena⁵, including the rejection of renal allografts in rabbits.⁶ It seemed reasonable, therefore, to study its effects in an animal model of bone marrow transplantation. Cs A permits the full establishment of a rat marrow graft across the major histocompatibility barrier. Given post grafting it prevents GvHD in an AgB mismatched donor-recipient combination and allows recovery of T-dependent immune functions as early as 28 d after transplantation. Thus, Cs A seems to be a promising agent for use in clinical marrow transplantation.

We have chosen an allogeneic rat system that we have found particularly useful as a preclinical model for human BMT⁷. Lewis rats (Ag-B1) were given a lethal dose of busulphan (BU), a drug that is not immunosuppressive in this species but myelotoxic, leading to the death of the animals from marrow failure unless protected with syngeneic marrow⁸. ACI (Ag-B4) marrow which is different at the MHC, will not be accepted by these BU-treated animals, unless they are immunosuppressed with high doses of Cy or rabbit anti-rat thymocyte serum (RARTS)⁹. If Cs A is substituted for Cy or RARTS and given as a daily injection of 25 mg per kg subcutaneously for 28 d, the animals will accept a bone marrow graft across the major histocompatibility barrier and become healthy chimaeras as evidenced by typing on day 21 (Table 1). The animals furthermore accept skin grafts from donor- and recipient-type animals (ACI and Lewis) but reject a third party (BN) skin graft. These data suggest that Cs A is able to provide the necessary immunosuppression to allow the establishment of a functional, histoincompatible marrow graft in an animal that suffers from a presumed lethal, BU-induced marrow aplasia.

An immunosuppressive drug like Cs A, that has been claimed to induce specific immunological tolerance¹⁰, should have a marked potential for the prevention and treatment of GvHD, a disease considered to result from an immunological attack of immunocompetent donor T cells against host target tissues¹¹. This disease can be studied with relative ease in the rat bone marrow transplantation model, where a clinical syndrome consisting of weight loss, marked dermatitis, diarrhoea and general unkempt appearance can be easily recognised, especially when a histoincompatible donor-recipient combination is used¹². All Lewis rats, conditioned with 1,000 rad TBI and infused with AgB-incompatible ACI marrow cells, developed severe GvHD within 12 d and showed a mortality of 75% (6 out of 8) by 35 d post-transplantation. Subcutaneous daily injections of Cs A starting 1 d post-transplantation and maintained until day 18 completely prevented the development of GvHD as judged by clinical observation, and by histological examination of the skin on days 14, 17, 21, 28, 35, and of tongue, liver and intestine on days 21 (Fig. 1), 28 and 35. These Cs A-treated animals have now survived more than 50 d post-transplantation without any signs of clinical GvHD and seem to

be in excellent health. The peripheral lymphocytes of these chimaeras were shown to be of donor (ACI) origin with the use of specific cytotoxic typing sera¹²; furthermore, they accepted donor- and recipient-type skin grafts but rejected third party (BN) skin allografts in a normal fashion, fulfilling the criteria for operational tolerance.

Borel *et al.*⁵ have used Cs A to affect GvHD in a rat bone marrow transplant model, and have shown that oral administration of Cs A merely delayed the onset of lethal GvHD in a parent-to-hybrid situation. The complete prevention of GvHD clinically and histologically seen in our study could be due to the different donor-recipient combination (although we used the most histoincompatible one available to us) but might alternatively be due to the higher doses of Cs A applied, the route of administration (parenteral compared with oral), or the duration of administration.

In previous studies¹³ we have evaluated the immune status of rat chimaeras transplanted with allogeneic histoincompatible bone marrow and noted a marked immunodeficiency. Such animals failed to demonstrate delayed type hypersensitivity (DTH) at 40 d post-transplantation and showed poor antibody responses to sheep red blood cells (SRBC)¹⁴. This defect has been described in other animal systems and in man¹⁵ and is

Table 2 Immune recovery of marrow transplant recipients after Cs A treatment

Donor-recipient combination	ACI*/Lewis	ACI*/Lewis, Cs A [†]	Lewis†/Lewis
Relative reconstitution of lymphoid tissue	(n = 4)	(n = 4)	(n = 4)
<i>Thymus</i>			
Cortex	all 1+	all 3+	3+, 3+, 3+, 4+
Medulla	all 1	all 2+	all 4+
<i>Spleen</i>			
T-cell region	all 1+	all 3+	3+, 3+, 4+, 4+
B-cell region	all 0	all 3+	all 4+
<i>Lymph node</i>			
T-cell region	all 1+	all 2+	3+, 3+, 4+, 4+
B-cell region	all 0	all 2+	3+, 3+, 4+, 4+
Delayed type hypersensitivity to DNCB	all 0	all 2+	all 3+
Antibody to SRBC (log ₂)§	all 1	4,4,4,5	6,7,7,7

Groups of Lewis rats (16 per allogeneic group, 8 per syngeneic group), were given 1,000 rads TBI and injected with 60×10^6 nucleated ACI* or Lewis† marrow cells i.v. One group of rats[†] was, in addition, given Cs A 25 mg per kg s.c. daily for 18 d starting 1 d after marrow infusion. Four animals out of each group were killed at day 21 (days 28 and 35 in the Cs A-treated group) and the lymphoid organs examined histologically. The relative number of lymphocytes in the lymphoid tissues are rated on a 0 to 4+ scale with 4+ being that of a normal Lewis rat. Surviving animals were immunised i.v. with 1 ml 10% solution of SRBC and the saline agglutination titres determined 1 week later. § In addition, the animals were sensitised to DNCB (600 µg ml⁻¹ over 1 cm² skin) on day 28, challenged on day 42 (60 µg ml⁻¹ over 1 cm² skin) and biopsied on day 44. The delayed type hypersensitivity reaction was graded on a 0-4+ scale, 4+ being the reaction of a normal Lewis rat.

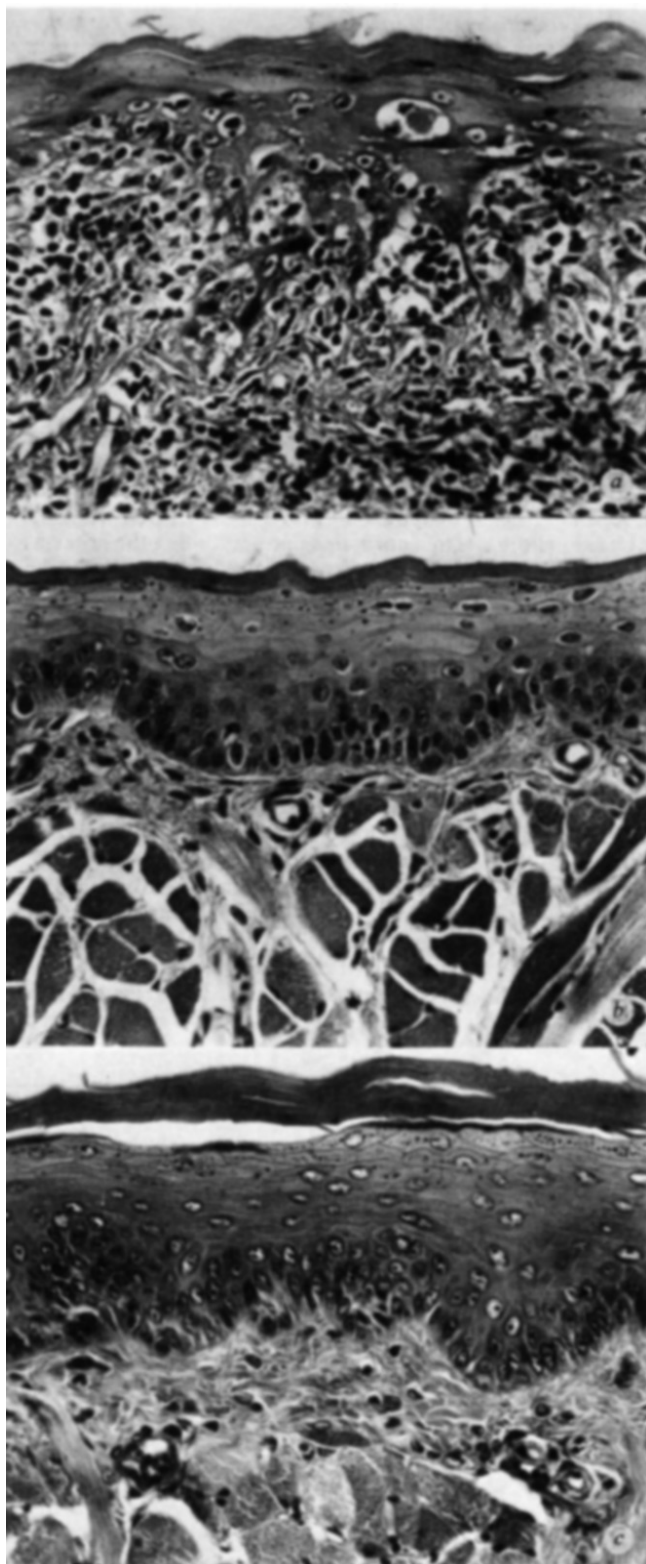


Fig. 1 Effect of Cs A on the development of GvHD in the rat. Lewis rats were given 1,000 rad total body irradiation (dual source ^{137}Ce small animal irradiator delivering 136 rad per min) followed in 24 h by infusion of 60×10^6 ACI (groups *a* and *b*) or Lewis nucleated marrow cells (*c*). Group *b* was treated in addition with Cs A given daily subcutaneously in a dose of 25 mg per kg from day +1 through day +18 post-transplantation. Four animals of each group were killed 28 d post-transplantation and autopsies performed. A representative section of the tongue is shown (haematoxylin and eosin, $\times 400$). A graft-versus-host reaction is recognised in the allogeneic group (*a*), which did not receive Cs A. No histological difference can be seen between Cs A-treated allogeneic chimaeras (*b*) and rats given syngeneic marrow (*c*).

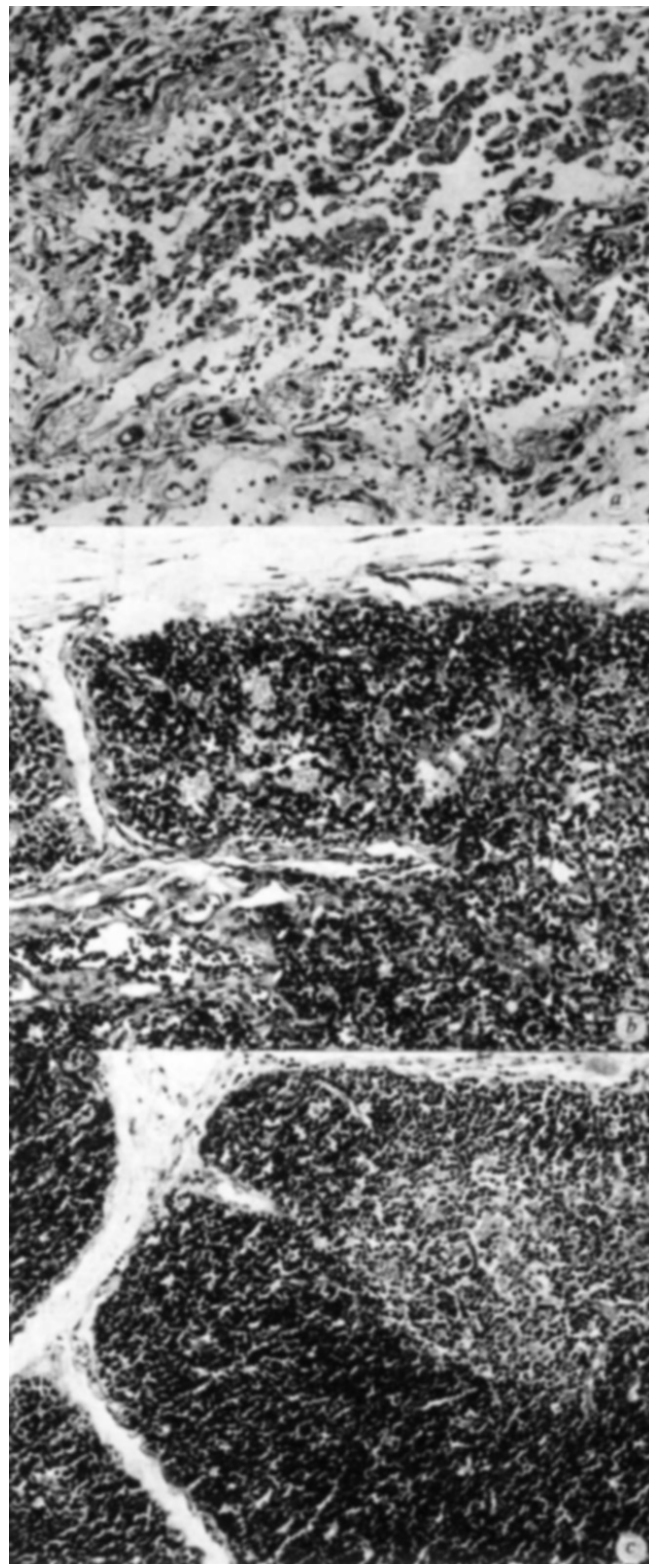


Fig. 2 Effect of Cs A on lymphocyte repopulation in the thymus of allogeneic marrow graft recipients. Lewis rats, conditions with 1,000 rad total body irradiation and infused with ACI (*a* and *b*) or Lewis (*c*) marrow, were either non treated (*a* and *c*) or treated (*b*) with Cs A (see Table 2). The thymuses of the animals were examined histologically (stained with haematoxylin and eosin, $\times 200$). Cs A-treated rat chimeras show good reconstitution of the thymic cortex (*b*), with medium and small lymphocytes, occasional lymphoblasts and mitotic figures. These lymphoid cells were shown by cytotoxic typing analysis to be of ACI (donor) origin. The untreated syngeneic recipients have reconstitution of the cortex with small lymphocytes and have a distinct corticomedullary junction.

considered to contribute to a significant degree to the impaired defence of the host against opportunistic infections post-transplantation¹⁶, especially those leading to an often fatal interstitial pneumonitis¹⁷. The aetiology of this immune defect is poorly understood, but might be related to graft-versus-host reactions, as the degree and length of immunodeficiency, post-transplantation correlate with the degree of histoincompatibility between donor and recipient¹⁸.

As GvHD was prevented in our rat model by Cs A we evaluated the immune status of the resulting chimaeras morphologically and functionally. Lewis rats were conditioned with TBI and given syngeneic marrow, allogeneic (histoincompatible ACI) marrow and allogeneic (ACI) marrow plus Cs A daily for 18 d, starting on day 1 post-transplantation. Representative animals out of each group were killed on day 21 (days 28 and 35 in the Cs A-treated group) and the lymphoid organs examined histologically. The striking repopulation of the T-cell areas in the lymphoid organs of allogeneic Cs A-treated chimaeras (Fig. 2) correlates with a recovery of immunological function (Table 2). Cs A-treated chimaeras immunised with DNCB and injected with SRBC were capable of mounting an immune response against SRBC and showed DTH, in striking contrast to the immunodeficient allogeneic chimaeras not treated with Cs A.

At present it is unclear how Cs A induces specific tolerance without interfering with the immune reconstitution. Studies by Green and Allison⁶ have shown that Cs A seems to act on lymphoblasts with both T- and B-cell markers and presumably eliminates or suppresses clones of lymphocytes responding to the antigens in question. The operational tolerance which we have in our Cs A-treated allogeneic chimaeras would then have been caused by a specific deletion or inactivation of clones of donor cells responding to host target antigens according to the clonal selection theory of transplantation tolerance. On the other hand, we and others have shown that transplantation tolerance might be induced and maintained by donor-type suppressor cells^{14,15,19,20}. It is tempting to speculate that Cs A acts by accelerating the appearance of suppressor cells, and indeed we have found in preliminary experiments that Cs A-treated allogeneic chimaeras possess cells in the spleen as early as 21 d post-transplantation, which suppress mixed lymphocyte reactions between donor- and recipient-type cells (data not shown). If these findings can be confirmed in future experiments, we might have a better understanding of the mechanism of action of this new drug and of the mechanism of transplantation tolerance as well.

Our studies seem to indicate that the optimism for Cs A generated from studies in other animal systems is justified. In our rat system this drug proved as immunosuppressive as Cy or RARTS, prevented GvHD and allowed early recovery of the immune function post transplantation. Cs A therefore seems to be a promising agent for use in clinical BMT.

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PETER J. TUTSCHKA*
WILLIAM E. BESCHORNER
ANTHONY C. ALLISON
WILLIAM H. BURNS
GEORGE W. SANTOS

Johns Hopkins Marrow Transplant Unit,
Oncology Center, and Department of Pathology,
Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205

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* To whom reprint requests should be addressed at Oncology Center, The Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, Maryland 21205.

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Degranulation capacity of cultured mast cells in the presence of histamine releaser

A PURE population of mast cells develops when suspensions of lymphoid cells prepared from mice pre-immunised with horse serum are grown on embryonic mouse skin fibroblast monolayers¹. These *in vitro* matured mast cells are capable of undergoing repeated cycles of degranulation and regeneration². Both IgG₁ antibody and the corresponding antigen and the compound 48/80 (ref. 3) trigger degranulation of more than 90% of the mast cells, releasing 80 to 90% of their histamine content². This produces an immediate effect on the cells in the fibroblast monolayer. The well-stretched cytoplasm is displaced, opening numerous 'windows' scattered over the whole monolayer². These capacities of cultured mast cells raise questions on the relationships between them, the degranulating agent and the degranulating products released into the medium. Using our tissue culture, mast cell behaviour can be studied in the continuous presence of the degranulating agent or of the degranulating products. We show here that mast cells regenerate degranulation irrespective of the continuous presence of the histamine releaser compound 48/80, but they become unresponsive to its degranulating effect, although they are totally degranulated by IgG₁ and the corresponding antigen.

The first experiments compared the behaviour of the mast cells grown: (1) in the continuous presence of 48/80 and the degranulating products; and (2) in similar cultures but from which the medium containing the 48/80 and the degranulating products was replaced 15 min later by a fresh 48/80-free medium. No difference was observed between the two groups. After 3-5 h, the fibroblasts stretch back, the windows close and the monolayer resumes its former confluent texture, with a large number of granules spread over it. The degranulated cells start immediately to synthesise histamine at an accelerated rate, and in 3 d the amount of histamine is totally replenished (manuscript in preparation). In some cases it even surpasses the level of the control undegranulated cultures (Table 1).

To see whether the regeneration capacity and lack of degranulation activity is due to decomposition of 48/80 we transferred medium containing 48/80 after further 4 days' incubation of the mast-cell cultures (91% mast cells degranulated, and 83% of the histamine was released 15 min after addition of the 48/80) to parallel control plates containing mast cells maintained with ordinary medium. Histamine was released and windows were formed (Table 1, group B). This clearly shows that 48/80 preserves its degranulating capacity over a period of 4 d. In further experiments 48/80 was transferred every 24 h for 4 d to new mast cell plates. At the fourth transfer the percentage of degranulated mast cells dropped from 73 to 45%. To confirm that the regenerated mast cells are refractory to triggering by 48/80, we replaced the 4-d-old medium with fresh medium

containing 48/80 and incubated the plates for 15 min. No degranulation occurred, and a negligible amount of histamine was found in the medium (Table 1, group A). However, when the medium containing fresh 48/80 was replaced by anti-horse serum IgG₁ plus antigen there was intensive degranulation (91%) with abundant wheal formation and 90.3% release of histamine (Table 1, group A).

This desensitisation course was studied further by exposure to serial dilutions of 48/80. Half of the plates of each dilution were assayed for histamine release after 20 min of exposure (Fig. 1), while the other half was incubated for an additional 3 d and then re-exposed to 15 µg 48/80 for 20 min (Fig. 2). There was complete unresponsiveness in cultures incubated with ≥ 10 µg per ml. (Cell counts showed that the unresponsiveness was not a result of diminishing number of mast cells in the cultures.) However, mast cells degranulated by 15 min incubation with 48/80 and then maintained further for 4 d with ordinary medium were degranulated thoroughly (87%) by fresh 48/80—like the control plates maintained with the ordinary medium alone (91% degranulation) (see also Table 2).

The persistence of refractoriness of regenerated mast cells after removal of 48/80 was examined by two methods. In the first method, cultures were exposed to 10 µg of 48/80 per ml (79.8% histamine release) either for 3 d, or for only 20 min to allow the degranulation to terminate followed by fresh 48/80-free medium for 3 d. Control cultures received only fresh

Table 1 Histamine release by 48/80 and IgG₁ in mast-cell cultures grown continuously with 48/80

Experimental group	Treatment of cultures	Free histamine (µg per plate)	Histamine in cells	Histamine release (%)
A	4 d with 48/80; old medium removed, fresh medium + 48/80 added for 15 min	0.72	8.44	7.9
	Anti-horse serum (IgG ₁) + horse serum	7.62	0.82	90.3
B	Recipient of old medium with 48/80 from group A	10.36	3.71	*
C	Maintained 4 d with 48/80	—	8.28	—
D	Maintained without 48/80	—	7.03	—

To obtain clonal differentiation of the mast cells, lymphoid cell suspensions (prepared from mice immunised with horse serum^{1,2}) were grown on fibroblast monolayers. BALB/c mice, 2–4 months old, were injected with 0.2 ml horse serum twice a week for 4 weeks. The animals were bled 6–12 d later and the serum was collected and frozen. This preparation, heated to 56 °C for 30 min to inactivate IgE, was used in inducing degranulation. The suspensions were prepared as described previously^{2,4}. Cells 10^7 in 2 ml Dulbecco's medium, supplemented with 15% heat-inactivated horse serum, were plated on 35-mm plastic Petri dishes containing fibroblast monolayers, prepared by plating 2.5×10^5 embryo skin cells in 2 ml Waymouth's medium supplemented with 10% calf serum and ready for use 4–6 d later. In these cultures mast-cell clones attained maximal growth at ~15–25 d. Cultures were normally passaged once or twice by trypsinisation between the 10th and 24th days, with the trypsinised cell suspension plated to make 2 new plates from each old plate. Within 4–6 d, mature mast cells spread confluent over the monolayer. On the 19th day of culture, 20 µg of 48/80 (sigma) in 2 ml Dulbecco's medium were added to half the plates while the other half received fresh medium only. 91% of the mast cells degranulated. The cultures were incubated further for 4 d. The plates maintained with 48/80 contained 1.1×10^6 mast cells and those without it 0.9×10^6 mast cells. Histamine was titrated by Snyder's radioenzymatic assay as described previously². The fluid, removed from the plate, was spun at 2,000 r.p.m. for 10 min. The supernatant was frozen pending assay of the free histamine in the medium; to the sediment, 2 ml of 0.1 M HCl was added and the whole content transferred to the plate containing the monolayer. The plates were frozen at -20 °C pending assay of the histamine in the cells.

*As the medium already contained histamine, the extent of degranulation is shown by the amount of histamine retained in cells.

Table 2 Degranulation capacity of mast cells in culture after 3-d-old 48/80-containing medium was removed

Time after removal of 48/80 (h)	3 days with 48/80		Without 48/80		15 min with 48/80	
	Second Challenge					
	48/80	IgG ₁	48/80	IgG ₁	48/80	IgG ₁
	Histamine release (%)					
0	25.1	76.4	70.2	—	65.1	78.2
2	26.3	—	82.3	—	74.5	—
4	26.0	80.4	84.4	70.6	—	—
8	45.3	—	—	—	—	—
16	39.1	—	—	—	—	—
24	45.0	78.9	79.0	75.4	66.4	78.2
48	27.3	—	75.4	—	65.9	—
72	33.5	71.5	80.0	60.0	76.6	70.6

Culture was established by plating 10^7 lymph node cells prepared from 4 month old BALB/c mice, 1 d after booster injection of horse serum. Culture was passaged 10 d later to make 3.2 new plates from each old plate. After 6 d, when there were 5.3×10^5 mast cells per plate, 20 µg of 48/80 in 2 ml Dulbecco's medium were added and the cultures were incubated for an additional 3 d. Then the medium was removed, cultures washed twice and fresh 48/80-free medium was added. At intervals the medium was removed and either 1 ml of Dulbecco's medium containing 10 µg 48/80 was added or 0.4 ml of antiserum (PCA titre in mice-1000), dilution 1:5 was added and followed 3 min later by 0.4 ml of 1% horse serum. After 20 min incubation the plates were processed for histamine assay.

medium. The old medium was then removed, cultures were washed twice and fresh 48/80-free medium was added. At intervals, 1 ml of medium containing 10 µg 48/80 or IgG₁ + 1% horse serum (see Table 1 legend), was added and the percentage of histamine release was determined. The results are shown in Table 2. In this culture, incubation with 10 µg 48/80 did not produce complete desensitisation (25% histamine release). Nevertheless, it can be seen that the responsive capacity was only slightly increased after removal of 48/80 (33% and 45% release). On the other hand, the response to IgG₁ was not affected by pretreatment with 48/80 and remained high in all the experimental groups.

In the second method mast cells were degranulated by 48/80 and then maintained further in the same degranulating medium for 3 d while control plates were maintained with ordinary medium. Both groups were passaged by trypsinisation to make 3.5 new plates from each older plate. (Note that trypsin releases about 70% of the histamine^{2,5}.) The degranulation capacity was

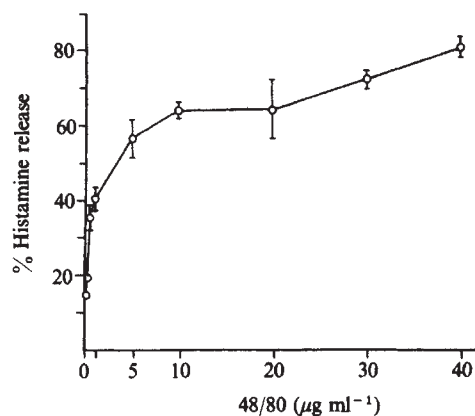


Fig. 1 Percent of histamine release as a function of dose of 48/80. A 16-d-old culture, 6 d after trypsin passage, making 3.2 new plates from each old plate. Counts of mast cells yielded an average number of 8.9×10^5 per plate. The cultures were incubated for 20 min before being processed for histamine assay.

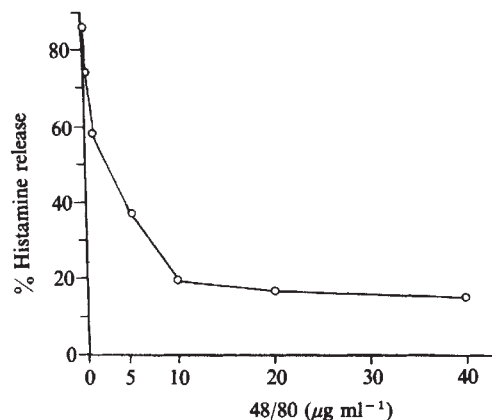


Fig. 2 Same culture as Fig. 1, but plates were incubated further for 3 d with the medium containing the various amounts of 48/80. These plates were then rechallenged with 15 μg 48/80 in 1 ml of medium. They were incubated for 20 min before processed for histamine assay.

determined immediately after plating in fresh medium and at different times thereafter. Results are shown in Fig. 3. At time zero histamine was not released, as compared to 10% in the control cultures; but the degranulation capacity gradually increased during the first 24 h, coinciding with the replenishment of the cellular histamine content until 48–72 h, when the response to 48/80 completely recovered. The study shows that without trypsinisation, restoration of the degranulation capacity was limited but virtually complete after such treatment. We presume that the refractoriness is induced by 48/80 which is bound to the cells during regeneration and detaches after trypsinisation.

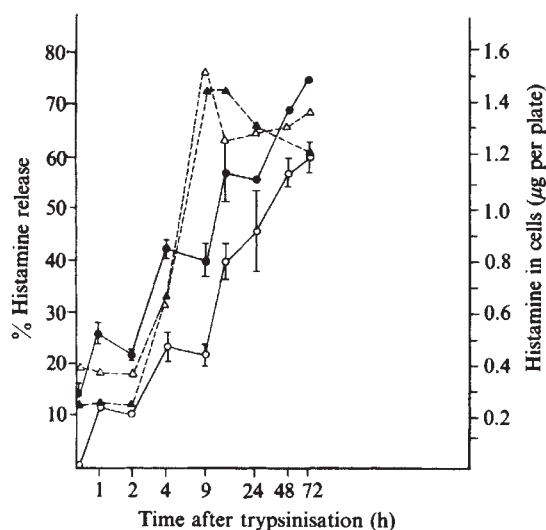


Fig. 3 Restoration of the degranulation capacity after passaging by trypsinisation and plating in 48/80-free medium. Culture was established by plating 10^7 lymph node cells prepared from 4-month-old BALB/c mice 14 d after last injection with horse serum. Culture was passaged 8 d later to make three new plates from each old plate. Seven days later, 40 μg 48/80 in 2 ml Dulbecco's medium were added to half the plates while the other half received fresh medium only. 65% of the histamine was released. The cultures were incubated further for 3 d. Then the medium was removed and trypsin solution (0.3% in Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline) was added. After 20 min the cells were collected, spun down, resuspended in Dulbecco's medium and horse serum and plated in 35-mm Petri dishes to make 3.5 new plates from each old plate. At different intervals, the medium was removed and 1 ml medium containing 15 μg 48/80 was added. After 20 min the plates were processed for assay of histamine. % Histamine release: $\circ$ — $\circ$ 3 d with 48/80; $\bullet$ — $\bullet$ 3 d without 48/80. Histamine in cells (μg): $\triangle$ — $\triangle$ 3 d with 48/80; ∇ — ∇ 3 d without 48/80.

This work has revealed hitherto unknown properties of the mast cell. The new information is important in view of its role in allergy. It is clear that in the continuous presence of the histamine releaser 48/80, the cell is reversibly desensitised, an effect which possibly comes under the heading of tachyphylaxis—development of tolerance to second exposure to a drug or an agonist^{6,7}. Obviously, it is still to be seen whether there is a similar response to reaginic antibodies and the corresponding allergens. Unfortunately, attempts to maintain mast-cell cultures in the continuous presence of mouse anti-horse serum plus horse serum as antigen, in dilutions optimal for total degranulation proved unsuccessful. This was probably because of the stimulation of a high metabolic rate with effects on the medium pH. A similar effect was obtained when such antibody-antigen complexes were added to fibroblast and macrophage cultures. This observation indicates recourse to classes and subclasses of purified immunoglobulin; thus, application of pure IgE, IgG₁, IgG_{2a} and IgG_{2b} to the different cells grown in our cultures would make it possible to assign the *in vitro* manifestation to its cause. This is important, as while both IgE and IgG₁ degranulate mast cells, they neither fix complement nor opsonise macrophages; by contrast, IgG_{2a} fixes complement and attaches to the Fc receptor on the macrophage⁸.

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HAIM GINSBURG
DORIT BEN-SHAHAR
ILAN HAMMEL
ESTHER BEN-DAVID

Department of Immunology, Faculty of Medicine,
Technion, Israel Institute of Technology,
Haifa, Israel

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Orderly compression of the retinotectal projection following partial tectal ablation in the newborn hamster

MANY hypotheses have been proposed to account for the development of the exquisitely specific systems of neuronal connections in the brain. Axons might read chemical labels on their target cells, attaching only to cells with a matching label¹; axon and target cell populations might arrange a match without cell-to-cell specificity, using such mechanisms as differential advantage in competition for terminal space^{2,3}; or some feature of the development and maturation of an axon and target system might produce ordered patterns of connectivity. The principal model system used to test these hypotheses has been the retinotectal projection of animals with nervous systems which are capable of regeneration, although it is not known whether this system in fact behaves like a developing nervous system. After removal of half the tectum in goldfish or frog, for example, an entire retina will compress its representation uniformly onto the remaining half tectum^{4,5}. We have repeated this experiment in the developing mammalian brain, and have found that following a partial tectal ablation in a neonatal hamster, the projection from the retina to the superior colliculus will terminate in an

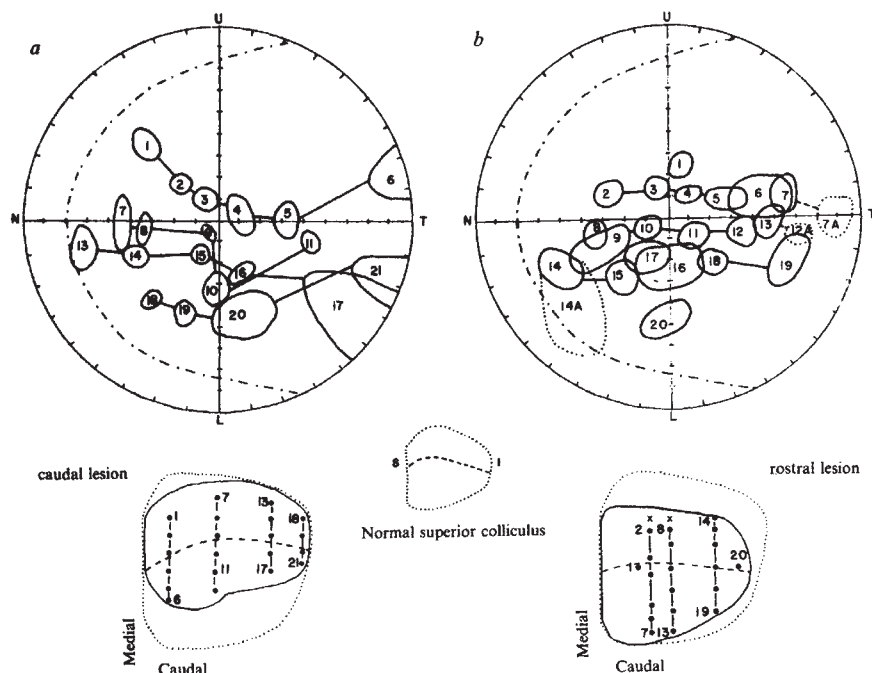


Fig. 1 Colliculi with reconstructed electrode penetrations and associated visual fields from a hamster with a neonatal lesion of the right caudal colliculus (*a*) and of the right rostral colliculus (*b*). The colliculi are shown in dorsal view; solid outlines indicate the remaining superficial gray layer in each hamster and dotted lines the normal extent of the superior colliculus. Electrode penetrations associated with receptive fields with defined borders are represented by black dots, and their corresponding numbered visual receptive fields are shown in the visual field diagram above. For visual convenience rostral to caudal series of penetrations and corresponding nasal-to-temporal visual receptive field are connected with lines. Medial colliculus, where upper visual field is represented, was not explored because of occlusion by the vein of Galen. The two penetrations marked by crosses were associated with diffuse receptive fields in the upper nasal quadrant including, but not limited to, the receptive field with the appropriate topographical position. The map of the visual field is centred about the projection of the optic disc. The broken line (---) indicates the perimeter of the visual field in normal hamsters. The nasal (N) to temporal (T) axis is determined

by the projections of the attachments of the medial and lateral rectus muscles, and is not the same as the horizontal plane defined by gravity. The orthogonal upper (U) to lower (L) axis is defined by the insertions of the superior and inferior rectus muscles. This superior-inferior axis, which divides the visual field into nasal and temporal halves, is shown projected on the surface of a normal superior colliculus (centre inset) and both damaged colliculi as a dashed line (---). Dotted receptive fields indicate fields found deep to the surface of the superior colliculus.

orderly way in a smaller than normal tectal volume, as in goldfish and frog^{1,4}. However, we have found preferential representation of nasal visual field which has not been reported in studies of non-mammalian animals in which the optic tract axons can regenerate. This may suggest some fundamental differences in developing and regenerating systems; in any case, it poses new problems for theories of neuronal specificity in developmental neurobiology.

On the day of birth, the brain of the hamster is quite immature. The first fibres from the retina have grown to the superior colliculus, entering the structure at its rostral border⁶. Although some axonal end arbors may already have begun to form near the tectal surface, major arborisation seems to begin around day 3, with the caudal, medial and lateral margins lagging behind rostral and central colliculus. An adult-like pattern and density is found by the age of 12–14 d. Partial lesions made in the colliculus on the day of birth thus intercept the initial stage of tectal innervation, and major reorganisations of retinal projection patterns do occur^{7–9}.

The superior colliculus is a prominent structure in the newborn hamster, and its superficial layers may be destroyed directly through the thin cranium by local application of heat^{7–9}. In 15 newborn hamsters partial lesions of the colliculus were inflicted on the day of birth by this method. After surgery, the animals were returned to the mother and allowed to mature normally. When these animals had reached the age of at least 2 months, the topography of the retinotectal projection was assessed electrophysiologically^{10,11}, and the extent of the neonatal lesion was determined thereafter by conventional histological reconstruction^{7–11}. In the 15 animals studied, the surface area of the remaining intact superior colliculus ranged from 40% to 80% normal, and tissue loss was confined principally to the superficial gray layer, as determined from sectioned material.

The receptive fields associated with a series of electrode penetrations of the surface of the superior colliculus in a hamster with a neonatal lesion of the caudal part of the superficial layer are shown in Fig. 1*a*. The entire visual field was represented at the tectal surface. Multiunit receptive field sizes reflected this compression of the visual field onto a smaller tectum. The average receptive field size in a normal hamster is $8.6 \pm 0.3^\circ$ (s.e. mean); in animals with caudal lesions average field size was

$12.4 \pm 0.4^\circ$. The topography was generally orderly, although localised mislocations or inversions were observed in this animal, as in every other. One striking anomaly was present in this and similarly prepared cases: the representation of the nasal visual field on the collicular surface was proportionately much greater than the representation of the temporal visual field (compare the positions of the superior-inferior meridian in the normal with the caudal lesion case in Fig. 1). The rostral-to-caudal extent of the nasal 70° of visual field was close to normal (75% normal length, as measured along the naso-temporal meridian). For the temporal 90° of visual field, the representation along the same meridian was only 48% of normal. This disparity in the rostro-caudal extent allotted to the nasal as opposed to the temporal visual field was present in all eight animals with caudal lesions. In three of the animals, in addition to the general compression, there was loss of 10–30° of visual field in the temporal periphery.

Lesions of rostral colliculus were made in a further seven newborn hamsters. The consequences of this lesion for axons entering the colliculus are somewhat different than the caudal lesion: axons entering the tectum must traverse an area of damaged tissue before innervating the remaining superficial gray layer. The mechanical disruption caused by this procedure may have contributed to a pattern of disorder in retinotopy observed in regions on the rostral border of the remaining superficial gray in four of the seven cases, an anomaly which has also been observed neuroanatomically^{7–9}. Receptive fields of units at the rostral border were large, encompassing whole quadrants or hemifields and were topographically disorderly, both in relation to neighbouring penetrations and throughout the depth of any particular penetration.

The compressed representation of the visual field observed after day-of-birth lesions of the rostral tectum was considerably more like normal in representation of nasal and temporal visual field than was observed in cases with lesions of caudal tectum. Nevertheless, when asymmetries occurred (three of the seven cases exceeded a 5% difference in representation of nasal visual field versus temporal visual field when compared to normal), nasal visual field was again preferentially represented. In Fig. 1*b*, the rostrocaudal extent of colliculus devoted to the remaining nasal field was 95% of normal; the extent devoted to temporal visual field was 70% of normal. The preferential representation

was never so extreme as that observed after caudal lesions. In no case, however, was there marked compression of the representation of nasal field relative to temporal field, even though the normal tectal terminal area for axons of retinal cells representing nasal visual field had been removed at birth.

An additional deviation from the normal representation of the retina in the tectum was observed in 12 of the 15 animals studied: extreme temporal visual field was represented both at the tectal surface and also deep to the tectal surface, lying directly ventral to receptive fields representing more nasal areas. In some cases, extreme temporal field could be found represented only deep below the tectal surface (Fig. 1b, dotted fields). This corresponds to an anatomical pattern observed previously⁷⁻⁹; in adult hamsters which had suffered caudal tectal lesions at birth, lesions of the nasal retina elicited axon terminal degeneration in deep superficial gray and in the upper part of intermediate gray in the caudal part of the remaining colliculus. These observations indicate an orderly topographic representation of the retina perpendicular to the surface of the superior colliculus which is never found in normal animals^{10,11}. Factors such as inappropriate angles of electrode penetration and reorganisation of collicular lamination, investigated thoroughly, could not account for these results⁷⁻¹⁰.

We do not know if the nonlinear compression of the visual field in the hamsters represents the stable end point of a process of compression, or a fixation of the map by maturation before a stable end point has been reached. In either case, the priority of nasal field representation in these compressed maps is an interesting clue to the mechanisms of map formation. The two most likely sources of this priority are features of development: the direction of retinal fibre entrance to the tectum from the rostro-lateral quadrant where nasal field is represented, and the sequence of maturation of retinal ganglion cells in hamster retina. We are presently investigating these two possibilities.

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BARBARA L. FINLAY
SUE E. SCHNEPS
GERALD E. SCHNEIDER

Department of Psychology,
Cornell University,
Ithaca, New York 14853
and

Department of Psychology and Brain Science,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received 25 October, 1978; accepted 21 May, 1979.

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A rich VIP nerve supply is characteristic of sphincters

VASOACTIVE INTESTINAL PEPTIDE (VIP), isolated from extracts of porcine duodenum by Said and Mutt¹, was at first thought to be a hormone². Immunohistochemical studies have since revealed that VIP has a neuronal localisation. VIP-containing nerves occur throughout the body, being particularly



Fig. 1 VIP-immunoreactive nerves (PAP staining) in smooth muscle of the feline pyloric sphincter (top) and duodenum (middle). Note the aggregation of coarse nerves in the sphincter region compared with the much lower number of immunoreactive nerves in the duodenum ($\times 250$). Bottom, VIP nerves (immunofluorescence) around the opening of the pancreatic duct in the papilla of Vater ($\times 150$).

frequent in the digestive tract³⁻⁷, the genitourinary tract⁸⁻¹⁰ and the upper respiratory tract¹¹. The nerves storing VIP or other neuropeptides (substance P, somatostatin and enkephalin) apparently represent additional types of autonomic nerves, distinct from the adrenergic and cholinergic ones. A great proportion of the peptidergic nerves seems to originate in nerve cell bodies located close to or within the innervated organ^{5-9,11}. The physiological significance of these new types of nerves is a matter of speculation, but a knowledge of their precise anatomical distribution will assist in defining their targets. We have previously observed that structures believed to exert a sphincter function receive a particularly rich supply of such nerves. We have now examined several sphincters, recognised or anticipated, and have established the presence of VIP nerves in all of them.

For chemical analysis specimens were taken from the mid and lower portion of the oesophagus, the stomach (cardia, antrum and pylorus) and the upper duodenum of seven cats of either sex. The material was immediately frozen and stored at -20°C until extraction in acidified ethanol and analysis by radioimmunoassay¹². For histochemistry material was collected from the above mentioned locations and from the sphincter of Oddi and the papilla of Vater from three cats. In addition, we collected specimens from the mid and lower portion of the ureter, from the bladder-urethra connection and from the bladder wall. From three male cats specimens were taken from the prostate, urethra and urethral collicles. The specimens were frozen to the temperature of liquid nitrogen in a propane-propylene mixture and freeze-dried. They were then exposed to formaldehyde gas at $+80^{\circ}\text{C}$ for 1 h (ref. 13) or to diethylpyrocarbonate vapour at

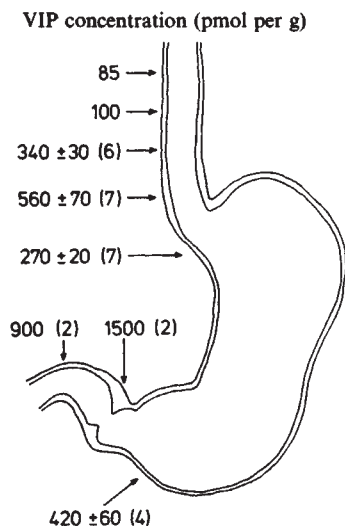


Fig. 2 Schematic drawing of the lower oesophagus, stomach and upper duodenum of the cat to show the distribution of immunoreactive VIP, measured by radioimmunoassay. Single determinations if not otherwise stated. The values for oesophagus and cardia refer to the whole wall, the values for antrum, pyloric sphincter and duodenum refer to smooth muscle only. Mean values $\pm$ s.e. (n).

+55 °C for 3 h (ref. 14) and embedded in paraffin. Sections were cut at 5 μ m and subjected to the indirect immunofluorescence method¹⁵ of the PAP staining technique of Sternberger¹⁶ for the demonstration of VIP, substance P or Leu-enkephalin. The VIP antiserum (code no. 5603) used in the radioimmunoassay was also used for immunostaining; it has been characterised in detail elsewhere^{12,17}. (For details on the use of the VIP, substance P or Leu-enkephalin antisera see refs 4, 18, 19.) The VIP antiserum was used in a dilution of 1:80 (immunofluorescence) or 1:5,120 (PAP staining), the corresponding values for the substance P antiserum dilutions were 1:40 or 1:160 and for the Leu-enkephalin antiserum 1:60 or 1:240. Controls were sections incubated with antiserum inactivated by the addition of excess antigen (pure porcine VIP, synthetic substance P or Leu-enkephalin; 10 μ g per ml diluted antiserum). Adrenergic nerves were demonstrated microscopically by fluorescence in the formaldehyde-fixed material¹³.

The region of the oesophago-gastric junction, pyloric sphincter, sphincter of Oddi and papilla of Vater received a very dense VIP nerve supply, more so than the smooth muscle of adjacent regions (Fig. 1). Immunochemical assay disclosed much higher VIP concentrations in the lower oesophageal and pyloric sphincter regions than in adjacent regions (Fig. 2). The openings of the ureters and of the urethra into the trigonum area of the bladder received a particularly rich supply of VIP nerves (see also refs 8, 9). By comparison, the body of the bladder and the ureters were sparsely innervated. Also, the smooth muscle surrounding the openings of the vasa deferentia and the prostatic ducts into the urethra were richly supplied with VIP nerves. In addition to VIP nerves the sphincter regions received adrenergic nerves and nerves displaying substance P (see also ref. 18) or enkephalin immunoreactivity. There were many enkephalin nerves in the lower oesophageal sphincter region and in the pyloric sphincter but not more than in adjacent regions; they were very few in the sphincter regions of the genitourinary tract. Acetylcholinesterase-positive nerves are very numerous in the sphincter of Oddi²⁰, whereas there are few adrenergic nerves in sphincters of the feline gut²¹. Generally, such nerves are not more numerous in sphincter regions than in adjacent regions. Subsequently, we have also found VIP-immunoreactive nerves to be comparatively numerous in sphincters of the guinea pig and rat. On the whole, however, the number of VIP nerves in smooth muscle is lower in these species than in the cat. In the rat immunoreactive-VIP nerves were

poorly demonstrated by antiserum 5603, but were easily demonstrated by another VIP antiserum (code no. 98P).

Thus, in the cat a very rich supply of VIP nerves is consistently found in the smooth muscle of all recognised sphincters and in some anticipated sphincters. We propose that a rich local supply of VIP nerves is characteristic of smooth muscle sphincters to the extent that an evaluation of the density of VIP innervation may assist in anatomically defining a sphincter. From what is known of the action of VIP on smooth muscle it seems likely that VIP is involved in sphincter relaxation²².

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J. ALUMETS O. SCHAFFALITZKY DE MUCKADELL
J. FAHRENKRUG F. SUNDLER
R. HÅKANSON R. UDDMAN

Departments of Histology and Pharmacology,
University of Lund,
Lund, Sweden

and
Department of Clinical Chemistry,
Bispebjerg Hospital,
Copenhagen, Denmark

Received 2 March; accepted 23 May 1979.

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Increased sensitivity to (+)amphetamine self-administered by rats following meso-cortico-limbic dopamine neurone destruction

DRUG self-administration (SA) in laboratory animals shows some of the characteristics of intracranial self-stimulation and of other natural reinforcers (for review see ref. 1). Animals will self-administer many of the same drugs abused by human subjects. Recent evidence has shown that (+)amphetamine reinforces behaviour in the same manner as conventional reinforcers¹⁻³ that it acts mainly on dopamine (DA) transmission, and that locomotor activation and stereotypy are mediated by the mesolimbic-mesocortical (A10 group) and nigro-striatal (A9 group) DA systems, respectively^{4,5}. Using various specific noradrenergic and dopaminergic blocking agents in a (+)amphetamine SA retention paradigm, DA transmission blockade was shown to produce effects similar to reward reduction and reward termination^{3,6,7}. DA transmission in the frontal-mesolimbic system has been implicated in brain stimulation

Table 1 Effects of (+)amphetamine injections (2 mg per kg, i.p.) on the locomotor activity of sham-operated rats and rats with VMT-A10 lesions

Groups	Locomotor activity (counts)			Time for maximal activation (min)
	1st 10 min	1st h	2nd h	
Control <i>N</i> = 6	131 ± 24	1,771 ± 225	1,178 ± 177	43 ± 5
VMT-A10 <i>N</i> = 8	291 ± 38*	1,283 ± 100	496 ± 101*	12.5 ± 1.6*

* The locomotor activity was measured in a circular corridor with photocell beams. The results are presented for each rat as the difference between (+)amphetamine and solvent scores.

reward⁸, and the link between self-administration of (+)amphetamine and reward mechanisms, both of which depend on DA transmission, may be related to the craving and early dependence that certain subjects display for addictive drugs. However, the mechanisms which lead to addiction are still unclear, and it is possible that in drug addicts the particularly strong and rapid addicting effect of the drug could be due to a pre-existing imbalance in some homeostatic mechanism. Thus, the use of healthy animals in a steady-state paradigm is not necessarily a good model for the study of the psychological or neurobiological vulnerability to drug addiction acquisition. Because the meso-cortico-limbic DA system may be an essential target for psychostimulant drugs, the lesion of this system could induce disruption of SA behaviour.

We report here that lesions of the ventral mesencephalic tegmentum (VMT) in the area of the meso-cortico-limbic DA cells (A10) induces paradoxically fast acquisition of intravenous self-administration of (+)amphetamine and also greater consumption of this drug.

Of 50 male adult Sprague-Dawley rats anaesthetised with ketamine, 30 received bilateral (± 0.4 mm) radiofrequency lesions of the VMT-A10 area surrounding the interpeduncular nucleus and in the remaining 20 rats (controls) the electrode was lowered 1 mm above the VMT area, and no current was passed. One month later, a silastic cannula, filled with a polyvinylpyrrolidone solution to prevent blood coagulation in the tubing, was chronically implanted in the jugular vein. The cannula was fixed with a stainless-steel connector on the calvarium. Three days later the SA experiments began. The rats were placed in an operant conditioning chamber with two levers situated at opposite sides of the cage. Depression of one lever activated a syringe pump that delivered 7.5 μ g per kg of (+)amphetamine in 2.5 μ l in 2 s for each press on a CRF schedule of reinforcement; depression of the other lever resulted in the same noises but led to no infusion. Each session (one every 2 d) lasted 12 h, from 1900 to 0700. The first session served as an operant control, during which the pump was disconnected, and the second and third sessions were the first and second (+)amphetamine sessions, respectively, and in the fourth session the (+)amphetamine was replaced by saline. Each rat was then killed under deep anaesthesia, the brain removed and the lesions examined histologically. Three rats with lesions considered to be unilateral were eliminated from data analysis. The lesions destroyed an area surrounding the interpeduncular nucleus and sometimes reached the medial part of the substantia nigra laterally and the ventral part of the nucleus ruber dorsally. Figure 1 inset shows a typical lesion.

The results are represented in Fig. 2. A two-factor analysis of variance was computed: lesioned against controls (A): $F = 53.7$, $P < 0.001$; (+)amphetamine against operant-saline (B): $F = 49.05$, $P < 0.001$; A $\times$ B interaction: $F = 17.55$, $P < 0.001$. The following remarks can be made. (1) There was no significant difference between the score of the two groups on either the operant or the saline session. (2) In the first (+)amphetamine session, the lesioned group's score was markedly greater than that of the control group ($t = 6.1$, $P < 0.001$), and different from

that of its operant session ($t = 10.8$, $P < 0.001$). There was a small but significant difference between the control group's score on the first (+)amphetamine session and on their operant session ($t = 2.05$, $P < 0.05$). (3) The second (+)amphetamine session confirmed the preceding results for the experimental group. The control group's drug session score differed statistically from that of its operant session ($t = 3.1$, $P < 0.001$). (4) The mean presses on the control lever (for the four respective sessions) were: control group, 8 ± 1 , 10 ± 4 , 13 ± 5 , 9 ± 2 ; VMT-A10 group, 7.5 ± 1 , 18 ± 5 , 17 ± 7 , 10 ± 3 . Typical acquisition and retention scores for a lesioned rat and a control rat are represented in Fig. 1, which shows that lever pressing for (+)amphetamine in the lesioned rat evolved dramatically, with an extreme sensitivity to the drug and enhanced acquisition of the operant response for (+)amphetamine.

The method used here was unusual because it focused on the acquisition of responding, a paradigm which may be particularly good for revealing pre-existing vulnerability to the drug. For this reason, a very small amount of the drug was available for each lever press, permitting only a low blood concentration at the beginning of the session. As the control operant behaviour scores of the two groups were identical, we can conclude that a different brain sensitivity to the drug determined the differing subsequent acquisition time courses. A lowered reactivity to (+)amphetamine in the VMT-A10 group can also be excluded as decreased sensitivity would contradict the rapid acquisition at very low blood concentration. The second clear modification of (+)amphetamine SA induced by VMT lesion is the intake of greater amounts of the drug. This may reflect a pathological need for the drug and a requirement for a higher blood concentration to obtain an identical 'rewarding' effect^{3,10,11}. These results must be compared with the relatively low score of the control group for the first (+)amphetamine session. In contrast to the gradual acquisition in control rats, the lesioned group acquired the response in less than 1 h, often less than 30 min and in some cases less than 15 min. During this time the total amount of the drug infused was less than 0.5 mg per kg, allowing us to eliminate motor preservation and stereotypy artefacts often observed in SA experiments¹². Moreover, although the number of presses on the control lever during the (+)amphetamine session was slightly enhanced, the animals did not show any apparent stereotypy.

The results are paradoxical, as previous data have shown that the blockade of DA release and re-uptake by (+)amphetamine are prerequisites for (+)amphetamine SA^{3,6,7}. Consequently, destruction of meso-cortico-limbic DA neurones, suspected to maintain (+)amphetamine reinforced behaviour, should have led to a disappearance of SA acquisition. To clarify this paradox we tested the reactivity and sensitivity to (+)amphetamine after

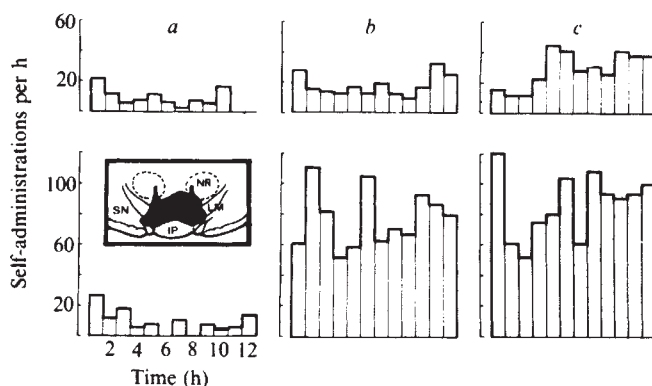


Fig. 1 Typical self-administration behaviour (0.75 mg per kg (+)amphetamine per 100 administrations) of a control rat (rat 5314, upper histograms) and a rat with bilateral VMT-A10 lesions (rat 5340, lower histograms). The lesioned area represented is typical of the lesions of the experimental group (IP, interpeduncular nucleus; SN, substantia nigra; NR, nucleus ruber; LM, lemissus medianus). a, Operant session; b, first (+)amphetamine session; c, second (+)amphetamine session.

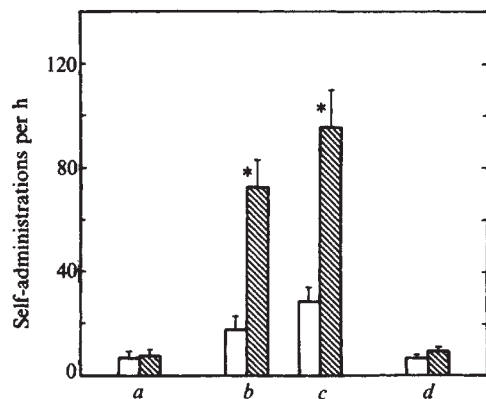


Fig. 2 (+)Amphetamine self-administration (0.75 mg per kg) (+)amphetamine per 100 administrations) in rats after radiofrequency lesions of the dopaminergic meso-cortico-limbic (A10) cell group lying in the ventral mesencephalic tegmentum (VMT) surrounding the interpeduncular nucleus (lesioned against controls: * $P < 0.001$, Student's t -test). a, Operant session; b, first (+)amphetamine session; c, second (+)amphetamine session; d, saline. □, Controls; n = 20; ▨ VMT-A10 lesioned; n = 27.

VMT-A10 lesioning. Eight VMT-A10-lesioned and six sham-operated rats, with no experience of the drug, received 2 mg per kg (+)amphetamine intraperitoneally (i.p.), and behavioural activation was measured in a circular corridor. Table 1 indicates that the lesion induced a 'hyper-reactivity' to (+)amphetamine, observed immediately after the injection. The maximal effect of the drug occurred at 12.5 min and 43 min, respectively, in the lesioned and control groups. However, it is also clear that the lesioned rats were 'hyposensitive' to the drug as the behavioural activation was observed for only 1 h in the lesioned group but lasted more than 2 h in the control group.

This second experiment may explain the SA behaviour of VMT-A10-lesioned animals. The hyper-reactive phase to (+)amphetamine could explain the rapid acquisition of SA, and the decreased time course could account for the high response rate (to maintain the rewarding effects of the drug). Our results clearly indicate that (+)amphetamine-induced behavioural activation is not completely blocked by our radiofrequency lesion of the VMT-A10 area, in contrast to the blockade obtained after massive destruction of DA-A10 terminals induced by 6-hydroxydopamine (6-OHDA) administration¹³. Therefore, it is probable that some DA neurones involved in (+)amphetamine-induced behavioural activation are still intact, that they are hyperactive, and that they react more strongly to (+)amphetamine but for a much shorter time. This hypothesis could explain the discrepancy between our results and previous data, which have shown that SA of psychostimulant drugs such as (+)amphetamine and cocaine are dependent on DA mechanisms; in these studies SA is suppressed by blockade of DA activity either by injecting a specific DA-receptor blocker^{3,6,7} or by total 6-OHDA destruction of DA-A10 innervation¹⁴. The hypothesis of incomplete destruction of DA neurones to explain (+)amphetamine SA effects could also explain the discrepancy between the behavioural syndrome induced by destruction of DA-A10 neurones after 6-OHDA injection into the terminal areas^{5,13} and the syndrome induced by lesion of the DA cell bodies. Radiofrequency lesion or local infusion of small amounts of 6-OHDA into the VMT-A10 area elicit a permanent behavioural perturbation characterised by locomotor hyperactivity and disruption of complex behaviour^{9,15-17}. This syndrome is correlated with depletion of DA but not of serotonin or noradrenaline¹⁸, and it may be due to a pathological state produced by incomplete destruction of VMT-DA neurones and overactivity of the remaining DA neurones. The syndrome is antagonised by chronic (+)amphetamine treatment or by very low acute doses of apomorphine¹⁹. These drugs shift the activity of the lesioned rats towards lower, normal levels. The possible existence of DA

receptors supersensitivity involved in reward mechanisms could explain the increased nucleus accumbens self-stimulation scores induced by radiofrequency lesion of the VMT area²⁰, while other studies using different lesion techniques demonstrated a decrease of this behaviour²¹. Our results may thus help in understanding the psychopathological disturbance which leads to drug addiction.

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MICHEL LE MOAL
LOUIS STINUS
HERVÉ SIMON

Laboratoire de Neurobiologie des Comportements,
Département de Neurobiologie,
Université de Bordeaux I,
Avenue des Facultés,
33405 Talence Cédex, France

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Extracellular Ca^{2+} and excitation-contraction coupling

THE role of external Ca^{2+} in excitation-contraction coupling has been controversial since Sandow¹ put forward the idea that contraction is initiated by an influx of Ca^{2+} on depolarisation. In the very thin lamellae (1 μm) of *Branchiostoma* myotome muscle ' Ca^{2+} -free' solutions with 1 mM EGTA block twitches completely^{2,3}. It was concluded that the Ca^{2+} influx during the action potential directly activates the myofilaments². In frog muscle twitches remain unchanged in 1 mM EGTA²⁻ (refs 4, 5). To ensure that $[\text{Ca}^{2+}]$ is sufficiently reduced in the narrow transverse tubular (T) system Barrett and Barrett⁶ raised the Ca^{2+} buffer concentration to 80 mM and observed that action potentials were no longer followed by twitches. In this letter we demonstrate that some further changes in the composition of the external fluids result in the restoration of contractions in both *Branchiostoma* and frog muscle indicating that a Ca^{2+} influx is not necessary for the initiation of contraction.

Single muscle fibres from the M. semitendinosus of the frog (*Rana temporaria*) were transferred into a Perspex chamber⁷ which allowed a quick exchange of solutions. Isometric contraction was measured with a force transducer⁵. To prevent a decrease in resting potential^{5,6} and an increase in external surface charge density⁸ at high EGTA concentrations, the solution included 5 mM free Mg^{2+} . The increase in ionic strength and osmolarity had no appreciable effect on the fibres. Figure 1 shows that in 80 mM free EGTA ($[\text{Ca}^{2+}] < 10^{-10}$ M) twitch

height decreased to 30% after 2 min. It then declined more slowly, reaching 5% after 2 h. Addition of 15 mM NaSCN caused a considerable recovery up to 30% of the initial level. The early fall in twitch force is probably due to an accelerated onset of inactivation, as in similar conditions the reduction in free Ca by as little as 2.5 mM EGTA²⁻, in the presence of Mg²⁺, causes a shift of the steady-state inactivation curve by 20–30 mV towards more negative potentials⁵. Our present finding that twitches did not disappear completely after a few minutes⁶ might be due to the presence of Mg²⁺, which prevents an increase in external surface charge density and thus a further shift of the inactivation curve beyond the resting potential. The continuous slow decrease in twitch force to very low values after the early fall might then be explained by assuming that (1) the final concentration of EGTA²⁻ in some part of the T-system is reached very slowly, (2) a continuous loss of Ca²⁺ from the muscle cell causes a depletion of internal Ca-stores, or (3) EGTA²⁻ has deleterious effects on the T-system.

However, the assumption that [Ca²⁺] in the T-system decreases only very slowly below the myoplasmic Ca²⁺ concentration¹¹ (~10⁻⁷ M) is very unlikely considering the SCN⁻ effect. This anion, which should not alter tubular free [Ca], causes a remarkable recovery of the twitch amplitude. It is known to shift the activation threshold to more negative potentials¹² without causing a corresponding shift in inactivation (our unpublished observations). This strongly suggests that a passive influx of Ca²⁺ during membrane depolarisation is not a prerequisite for activation.

The possibility that loss of internal Ca²⁺ and/or secondary effects are mainly responsible for the slow decline in force under high EGTA is also unlikely, as shown by the nearly complete recovery from EGTA²⁻ application and from the tetanus results. In the experiment shown in Fig. 1 tetanus force fell to 95% and 23% after 40 and 120 min, respectively, but rose again to 68% in SCN⁻. The tetanus/twitch ratio increased from 2:1 in normal conditions to about 10:1 in high EGTA. In one of the 11 experiments it reached 55:1. The increase in tetanus/twitch ratio shows that the coupling efficiency improves during repeti-

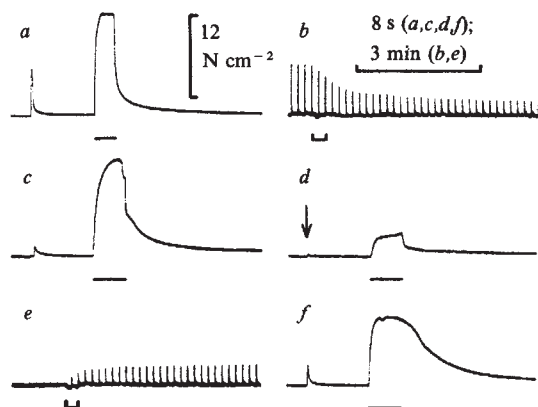


Fig. 1 Effect of high EGTA and SCN⁻ on twitches and tetani (50 Hz; indicated by horizontal bars) of an isolated skeletal muscle fibre (diameter 130 μ m). *a*, Ringer solution with 1.8 mM Ca²⁺; *b*, change from modified Ringer (see below) to a solution with 80 mM free EGTA ([Ca²⁺] $\leq 10^{-10}$ M) and 5 mM free Mg (96.2 mM total EGTA and 21.2 mM total Mg). The free concentrations were calculated as described by Portzehl, Caldwell and Rüegg⁹ using the absolute binding constants given by Sillén and Martell¹⁰ and assuming a Ca²⁺ contamination of 10⁻² mM. *c* and *d*, Twitches and tetani after 40 and 120 min in high EGTA, respectively. *e*, Change from high EGTA to high EGTA plus 15 mM SCN⁻, *f*, after 6 min in the latter solution. The brackets show the time needed for the exchange of solutions. In order to avoid a transient depolarisation beyond the contraction threshold after the replacement of NaCl in Ringer by Na-EGTA we exchanged 50 mM NaCl of Ringer by 50 mM Na propionate 10 min before application of EGTA²⁻. All solutions contained 2.5 mM KCl and 5 mM MOPS⁻ as buffer, pH = 7.0, T = 22–23 °C.

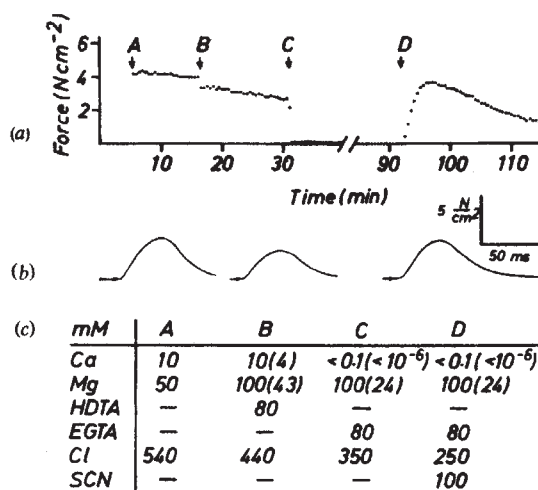


Fig. 2 Effect of high EGTA and SCN⁻ on twitch amplitude of a myotome strip from *Branchiostoma*. Diameter 170 μ m. Stimulating frequency 2 per min. *a*, Twitch amplitude in different solutions. *b*, Time course of single twitches. *c*, Composition of solutions in order of application (mM) with free concentrations of Ca and Mg in brackets. All solutions contained in addition (in mM): Na⁺ 400, K⁺ 10 and Tris (as buffer) 10, pH = 8.0; T = 10 °C. Since 80 mM EGTA replaces a considerable amount of Cl⁻, HDTA (hexamethylenediamine-*N,N,N',N'*-tetra-acetic acid) was added before application of EGTA to moderate the depolarising action of Cl⁻ reduction. This substance has a comparable binding characteristics for Mg²⁺ but possesses a 10⁶ times smaller absolute binding constant for Ca²⁺ than EGTA. Because very low concentrations of free Mg led to spontaneous twitches and contractures in Ca²⁺-free solutions a drastic reduction in free Mg was prevented in sol. B–D by increasing the total concentration to 100 mM. A large part of Mg²⁺ was reversibly bound to EGTA, thus reducing free EGTA but not its capacity to buffer [Ca²⁺] below 10⁻⁹ M.

tive activity, probably by a 'normalisation' of the inactivation kinetics⁵. In this context it should be noted that the coupling process improves, although internal [Ca²⁺] and thus the outwardly directed driving force for calcium ions increases. Thus it is likely that a further acceleration of inactivation is mainly responsible for the slow decline in twitch force in high EGTA.

For experiments with myotomes of *Branchiostoma lanceolatum* muscle strips, 1.5 mm long and 0.1–0.4 mm thick, were dissected and isometric twitches were elicited by short supramaximal current pulses. Figure 2 shows twitch amplitude in solutions of different composition. The pretreatment with HDTA (see legend to Fig. 2) caused a small decrease in twitch height, which might be due to the concurrent decrease in free calcium. When, however, external [Ca²⁺] was drastically reduced by high EGTA (solution C) twitch amplitude fell to very low values within 1 min. After 1 h, when twitches were no longer visible, 100 mM Cl⁻ was replaced by SCN⁻ (solution D). Subsequently twitch height increased almost to the original value within a few minutes. Thereafter, force gradually declined again, reaching 1/3 after 30 min.

Apart from a prolongation in latency time by about 100%, the time course of twitches in EGTA²⁻ + SCN⁻ was very similar to the control twitches (Fig. 2*b*). This again suggests that SCN⁻ simply strengthens the natural coupling process.

In our experiments we used 80 mM EGTA²⁻ to reduce the concentration of free Ca²⁺ below 10⁻¹⁰ M. Even if we assume that the actual Ca²⁺ concentration in the T-system or extracellular clefts remains 2–3 orders of magnitude higher than in the bulk solution it still should be below that measured in normal conditions in the myoplasm. In agreement with recent conclusions drawn from experiments with the calcium indicator dye arsenazo III¹³ we therefore believe that an influx of Ca²⁺ is not a prerequisite for the activation of contraction. Models¹⁴ which directly combine the Ca²⁺ release from the sarcoplasmic

reticulum with the membrane potential of the T-tubular membrane seem more appropriate to explain excitation-contraction coupling.

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WILTRUD SPIECKER
WERNER MELZER
HANS CHRISTOPH LÜTTGAU

Department of Cell Physiology,
Ruhr-University Bochum,
D-4630 Bochum 1, FRG

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Parallel regulation of acetylcholinesterase and pseudocholinesterase in normal, denervated and dystrophic chicken skeletal muscle

In vertebrate skeletal muscle acetylcholinesterase (AChE, EC 3.1.1.7) is usually considered to be involved in the termination of impulse transmission by hydrolysis of acetylcholine¹. AChE is often accompanied by a second enzyme², pseudocholinesterase (ψ ChE, EC 3.1.1.8), which differs from AChE in substrate specificity and in susceptibility to anticholinesterases^{3,4}. No role comparable with that of AChE in synaptic transmission is known for ψ ChE, despite its abundance at some sites and its occurrence (as shown histochemically) with AChE in various muscle endplates^{1,5}. It has been suggested that ψ ChE is a precursor of AChE in the superior cervical ganglion^{6,7}. Moreover, in ganglia and skeletal muscle of rats, forms of ψ ChE have been demonstrated⁸ homologous with forms of AChE observed previously⁹⁻¹¹. Elevated levels of AChE have been demonstrated in aqueous extracts of fast-twitch muscles of dystrophic as compared with normal chickens^{12,13}; ψ ChE levels increased similarly¹³, and also in such extracts of denervated muscles of normal chickens¹⁴. We show here that the total amounts and forms of AChE change in parallel with those of ψ ChE during development of skeletal muscles of both normal and dystrophic chickens, as well as on denervation of normal muscle. Our data strongly indicate that in these tissues the two enzymes are controlled by a common regulatory mechanism.

It has been reported^{12,15} that embryonic chick muscle has high AChE concentrations which fall during maturation, but those studies used conditions of no or low salt or detergent, which are now known^{8,11,16,17} to give low AChE extraction. We have homogenised the tissues in 1% Triton X-100/1M NaCl, shown¹⁷ to extract 95-100% of the total muscle AChE, and find (Table 1) that in the pectoral muscle of normal chickens AChE

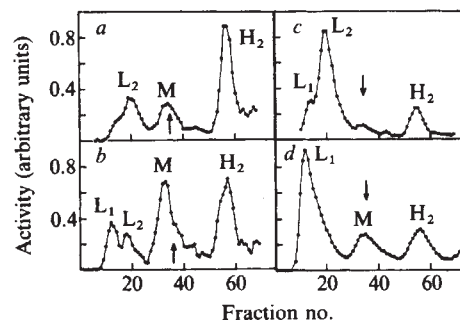


Fig. 1 Sucrose gradient centrifugation profiles of AChE and ψ ChE activity in extracts of pectoral muscle of normal (line 412) and dystrophic (line 413) chickens. *a*, AChE in muscle of a normal 19-d embryo; *b*, ψ ChE in muscle of a normal 19-d embryo; *c*, AChE in muscle from a 32-d dystrophic chicken; *d*, ψ ChE in muscle from a 32-d dystrophic chicken. Centrifugation was carried out on 5-20% sucrose gradients as described previously¹⁹, using extracts obtained as described in Table 1. AChE was assayed using 0.75 mM acetylthiocholine after 30 min preincubation with 10^{-4} M isoOMPA. ψ ChE was assayed using 0.75 mM butyrylthiocholine in the presence of 10^{-5} M BW 284C51. The arrow denotes the position of a catalase marker, taken as having sedimentation coefficient 11.4S.

levels are strikingly reduced in 1-month-old, as opposed to embryonic birds, and ψ ChE levels follow a similar course. In dystrophic birds, an initial decrease in AChE levels in pectoral muscle is soon followed by a dramatic increase with onset of the disease, and again the ψ ChE levels increase in parallel. Also, in the slow tonic anterior latissimus dorsi (ALD) muscle, which is not affected by dystrophy, significant (and similar) amounts of ψ ChE occur with AChE in both normal and dystrophic muscle. Table 1 also shows that following denervation of another fast-twitch muscle, the posterior latissimus dorsi (PLD), a 30-fold elevation in AChE levels occurs in 3-week-old birds, accompanied by a >20-fold elevation in ψ ChE levels.

Sucrose gradient centrifugation of extracts of chicken skeletal muscle reveals^{18,19} several molecular forms of AChE which are principally concentrated in three zones of the gradient, arbitrarily designated as heavy (H_1 , 16-20S), medium (M , 10-13S) and light (L_1 , 4-7S). It has recently been demonstrated that the H_2 form (~20S) predominates in normal adult pectoral and PLD muscles^{17,19}, that the L_2 (~6.5S) form is greatly elevated in dystrophic fast-twitch muscle^{17,19-21}, and that in young or embryonic pectoral muscle increased levels of M (~11S) and H_1 (~16S), as well as L forms are observed^{17,19}. Figure 1*a* shows a typical sucrose gradient profile for AChE obtained with an extract of embryonic muscle, displaying the L_2 , M and H_2 forms as the dominant components. Figure 1*b* shows a profile for ψ ChE in the same tissue extract. Activity peaks of ψ ChE are observed in the H , L and M regions of the gradient, as for AChE. The principal difference is that a considerable part of the activity in the L region corresponds to an L_1 (~4S) component. In the case of AChE the L_1 form is seen in large amounts only when protease inhibitors (always used here; see Table 1) are omitted from the extraction medium¹⁹. L_1 AChE thus seems to be produced by proteolytic degradation of L_2 , which is apparently a dimer of L_1 (ref. 22). Figure 1*c* shows that in adult dystrophic pectoral muscle the L_2 component of AChE is considerably larger than the H_2 component, with the M species making only a minor contribution. As shown in Fig. 1*d*, in the dystrophic adult muscle ψ ChE activity is also present in all three regions of the gradient. Again, the light forms are elevated, with the L_1 form predominating, but the M and H_2 forms are also prominent. The ψ ChE activity in the gradients displayed in Fig. 1*b, d* was assayed using butyrylthiocholine in the presence of the selective AChE inhibitor⁴, BW 284C51. When butyrylthiocholine and BW 284C51 were supplemented with the selective ψ ChE inhibitor⁴, tetra-*iso*-propyl pyrophosphoramidate (*iso*OMPA), no activity was observed in any part of the gradient. This clearly establishes that all peaks observed represent ψ ChE. It should also be noted that each ψ ChE peak seems to be fractionally

lighter (by 0.5–1S) than its corresponding AChE form. Thus, L₁, L₂, M and H₂ for ψ ChE have on average $s_{20,w}$ = 4.0, 5.9, 10.5 and 19.1 S, respectively.

The content of ψ ChE in the fast-twitch muscles of normal chickens is very low at 28 d of age (Table 1) and later. Nevertheless, this enzyme was shown (ref. 17 and additional unpublished data) to be a real component of both fast-twitch and slow tonic muscles of mature normal chickens, by the use of prolonged enzymatic reaction incubations of similar sucrose gradients: their pectoral and PLD muscles (at 32–100 d, perfused to remove blood ψ ChE) showed significant L ψ ChE (mainly L₂) and less, but distinct, M and H ψ ChE. In their ALD muscles, L₁ and L₂ are higher, with some M (and a trace of H) ψ ChE also present; the pattern is as shown¹⁹ for AChE there. On maturation of normal fast-twitch muscle, therefore, the parallel control of ψ ChE and AChE forms is lost, but it remains in the mature slow tonic muscle.

Vigny *et al.*⁸ reported only minute amounts of an H form of ψ ChE in rat supracervical ganglia and could not demonstrate any H form in mature skeletal muscle. It is thus particularly interesting that, using embryonic or dystrophic birds, we can demonstrate considerable ψ ChE activity in the H region, as the H forms of AChE are those which are ascribed to the endplate region in skeletal muscle^{9,10,18}. Consistent with this occurrence of the H forms of AChE at the endplate region is their appearance on innervation and their loss on denervation^{18,21}. It was, therefore, important to see whether the H form of ψ ChE would also disappear on denervation. This was investigated in the tonic ALD muscle of very young (~7-d) chickens, where the H forms of both AChE and ψ ChE are quite prominent (Fig. 2a, c). We confirmed that the H₂ form of AChE is largely eliminated within 7 days, with a concomitant increase in amounts of the lighter forms of the enzyme (Fig. 2b). The H₂ form of ψ ChE is similarly reduced (Fig. 2d), and, as in the case of AChE, increased amounts of the lighter forms of the enzyme are observed. A similar denervation experiment (not shown) on the PLD muscle of a mature dystrophic bird also demonstrated disappearance of the prominent H peak of ψ ChE together with disappearance of the corresponding AChE peak.

The role of the motor nerve in controlling muscle differentiation is especially pertinent to attempts to understand whether a given myopathy is of neurogenic or myogenic origin. In the case of inherited muscular dystrophy in humans, as well as in chickens and other experimental animals, this issue is extremely controversial²³. Although AChE levels increase in chicken

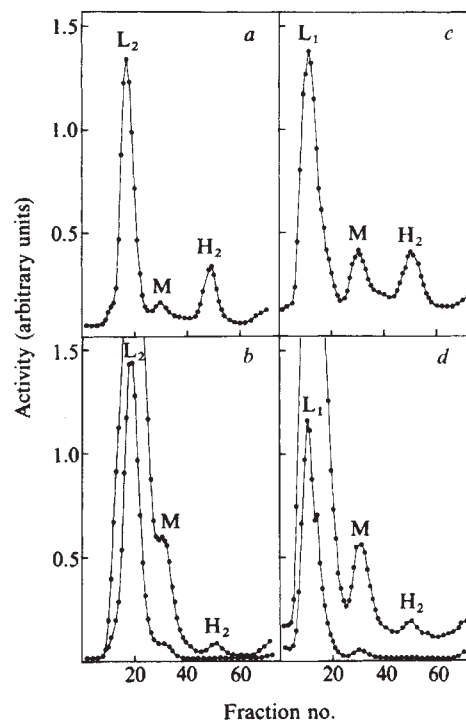


Fig. 2 Sucrose gradient profiles of AChE and ψ ChE activity in extracts of innervated and denervated anterior latissimus dorsi (ALD) muscles. Leghorn chickens were denervated on day 1 post-hatch as described in Table 1, and killed on day 7 together with control birds. *a*, AChE in control bird; *b*, AChE in denervated bird; *c*, ψ ChE in control bird; *d*, ψ ChE in denervated bird. Extracts were made as in Table 1, gradients were run as in Fig. 1 and assays carried out as in Fig. 1 except that BW 284C51 was omitted in the assay of ψ ChE. In the case of denervated muscle (*b*, *d*) the upper profile, in each case, was obtained by developing the gradient for the same time as for the corresponding control muscle (*a*, *c* respectively); the lower profile represents incubation for a much shorter time so as to reveal the relative magnitudes of the L peaks.

dystrophic muscle as in normal denervated muscle, this does not imply that the control of AChE levels is strictly neurogenic, as ACh receptor levels increase after denervation but not during dystrophy²⁴, and limb-bud transplant evidence has suggested that dystrophy in chickens is of myogenic origin²⁵. Moreover, whereas after denervation the H₂ form of AChE is virtually eliminated (see Fig. 2 and refs 18, 21), in the dystrophic condition it is elevated^{17,19–21}. Whatever the primary lesion in the dystrophy, our data show that it affects the levels and forms of ψ ChE similarly to those of AChE.

Because AChE and ψ ChE show large differences in binding properties^{3,4}, and no immunological cross-reactivity⁸, we presume they are products of different genes. For human serum ψ ChE a separate locus has, in fact, been established by analysis of genetic variants, including an inherited deletion of only this enzyme²⁶. Consistent with the concept of separate genes for muscle AChE and ψ ChE, the two enzymes do not always vary in parallel (in maturation of normal fast-twitch muscle). Thus, the present finding, that in several different states their various forms do change in parallel, is best explained by a regulatory mechanism that is common to the two genes. The close homology of the molecular forms of AChE and ψ ChE suggests that they are assembled into functional species by a similar mechanism. This is particularly remarkable in the case of the H form, where current evidence with respect to AChE supports the concept of a complex structure in which the catalytic subunits are attached to a collagenous tail^{16,22,27} which anchors the enzyme to the basal lamina²⁸.

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Table 1 Amounts of acetylcholinesterase and pseudocholinesterase in extracts of fast-twitch muscle from normal, denervated and dystrophic chickens

Muscle	Normal		Normal, denervated		Dystrophic	
	AChE	ψ ChE	AChE	ψ ChE	AChE	ψ ChE
Pectoral, 19 d <i>in ovo</i>	8.4	0.7			5.8	0.6
Pectoral, 32 d post-hatch	0.15	<0.01			1.9	0.6
PLD, 28 d post-hatch	0.88	<0.01	26	0.24		
ALD, 32 d post-hatch	3.90	0.27			2.26	0.20

Pectoral and ALD muscle samples were dissected from freshly killed embryos or 32-d-old chickens (normal [line 412] or dystrophic [line 413] from the University of California flock). Posterior latissimus dorsi (PLD) muscles were dissected from freshly killed normal Leghorn chickens. Denervation was carried out 7 d before sectioning the main nerve trunk at the brachial plexus. Tissue extracts were prepared as described previously¹⁹, in a medium containing protease inhibitors which had the following composition: 5 mM *N*-ethylmaleimide, 2 mM benzamide, 10 mM EGTA, 20 μ g ml⁻¹ pepstatin, 40 μ g ml⁻¹ leupeptin, 1 mg ml⁻¹ bacitracin, 1% Triton X-100, 1 M NaCl, 0.01 M phosphate, pH 7.0. AChE was assayed on 0.75 mM acetylthiocholine as described previously¹⁹, after preincubation with 0.1 mM iso-OMPA for 40 min to permit full inactivation of ψ ChE⁴. ψ ChE was assayed using either 0.75 mM acetylthiocholine in the presence of 5 $\times$ 10⁻⁵ M BW 284C51 (pectoral and ALD) or 0.75 mM butyrylthiocholine (PLD). The figures shown represent units per g tissue, where 1 unit is the amount of enzyme hydrolysing 1 μ mol of substrate per min in the assay conditions used.

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I. SILMAN*
L. DI GIAMBERARDINO†
J. LYLES
J. Y. COURAUD†
E. A. BARNARD‡

Department of Biochemistry,
Imperial College,
London SW7, UK
and

† Département de Biologie,
CEA de Saclay,
BP No. 2, Gif-sur-Yvette, France

Received 9 January; accepted 23 May 1979.

* Present address: Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel.

‡ To whom correspondence should be addressed.

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Human embryonic haemoglobins Gower 1 and Gower 2

THE human embryonic haemoglobins (Hbs) Gower 1 and Gower 2 (ref. 1) are present in fetuses up to 60 mm crown-rump measurement². However, relatively little is known about the structure and function of these haemoglobins because of the difficulty in obtaining suitable material, particularly since the introduction of the suction method for terminating pregnancy in the first trimester. Here we present the amino acid composition and sequence data for the human ϵ -chain, prepared by purifying Hb-Gower 2 ($\alpha_2\epsilon_2$) from early abortion samples. In addition, separation of embryonic red cells from the majority of the maternal red cells has led to the isolation of Hb-Gower 1 and the demonstration that it has the composition $\zeta_2\epsilon_2$.

Material was collected from vacuum abortions of 6–10 weeks gestation carried out by the standard technique for unrelated indications. The sample was initially passed through a coarse sieve to remove any tissue fragments, and then through a 20- μ m

pore-size filter. The red cells were packed and washed three times with isotonic saline, then lysed with water and the membranes extracted with carbon tetrachloride. These haemolysates contained only small amounts of Hb-F, and Hb-Gower 1 or Hb-Gower 2 were detected in only a few samples by starch gel electrophoresis.

The first step in the purification was the removal of Hb-A using a modification of the chromatographic method described by Huisman and Dozy³. The haemolysates were equilibrated against 0.05 M Tris-HCl, pH 8.05 $\pm$ 0.05, and loaded at room temperature on to a DEAE-Sephadex column (3 $\times$ 30 cm) equilibrated against the same buffer. The haemoglobins were eluted with pH 8.05 buffer and the first haem-containing peak collected; this included Hb-Gower 2, Hb-A₂, small amounts of Hb-A and some carbonic anhydrase. This fraction was concentrated and dialysed against developer A consisting of 0.2 M glycine and 0.01 g dl⁻¹ potassium cyanide, pH 7.4–7.7, and loaded at room temperature on to a column (1.5 $\times$ 25 cm) of microgranular DEAE-cellulose DE52 equilibrated with developer A (ref. 4). Hb-Gower 2 was eluted using developer A only, all other haemoglobins remaining firmly attached to the cellulose in these conditions. The Hb-Gower 2 was checked for purity by measuring the A ratio 280/345 nm of each fraction, and only those uncontaminated (ratio <1.5) with non-haem proteins were concentrated and used.

The electrophoretic mobility of Hb-Gower 2 was similar to that described previously¹. Spectral analysis between 250 and 700 nm of the oxygenated purified Hb-Gower 2 showed only minor differences when compared with a solution of oxyHb-A. Sephadex G-100 gel filtration⁵ gave a value of 30,000 for the molecular weight of Hb-Gower 2 and 45,000 for Hb-A, presumably indicating that the tetramer dissociates into $\alpha\epsilon$ dimers more readily than does Hb-A in similar conditions. SDS-polyacrylamide gel electrophoresis of Hb-Gower 2 in a concentration gradient of acrylamide⁶ that just resolved α - from β -chains, gave a broad band, the fastest portion corresponding to the α -chains of Hb-A with an overlapping zone between the α - and β -chains. There was no electrophoretic resolution of the α - from the ϵ -chains after treatment of the protein with *p*-chloromercuribenzoate (PCMB)⁷. Attempts to separate the globin chains using chromatography on CM-cellulose in 8 M urea⁸ yielded a single protein peak consisting solely of α -chains; the ϵ -chains could not be eluted from the column even using much stronger buffers and a higher pH. Separation of the globin chains was achieved by electrophoresis on cellulose acetate in 6 M urea, pH 6.4 (ref. 9), the ϵ -chains migrating anodally, close to the α -chains. However, this method was not suitable for preparative purposes. Further analysis was therefore carried out on whole globin prepared from purified Hb-Gower 2 by acid/acetone precipitation.

Tryptic peptide maps prepared by standard techniques⁸ showed many ninhydrin-positive spots immediately recognisable as peptides from the α -chain, but at least 10 were thought to be peptides from the ϵ -chain; all peptides were eluted with 6 M HCl for amino acid analysis. From the data obtained it was concluded that the ϵ -chain is similar to both the β - and γ -chains, and using tables from Dayhoff¹⁰ a sequence of the first 10 tryptic peptides (residues 1–87) of the ϵ -chain was constructed by comparison with known mammalian β -chain sequences. Six milligrammes of Gower 2 globin were also analysed in a Beckman model 890C protein sequencer. The data confirmed the sequence of the first 40 residues (Fig. 1).

Throughout these procedures no peptides belonging to the second half of the ϵ -chain were demonstrated. Presumably, these had precipitated as an insoluble core of undigested globin which was not detected in the small amount of material used. Cyanogen bromide digestion of Gower 2 globin followed by tryptic digestion and peptide mapping of the fragments separated by Sephadex chromatography showed the characteristic C-terminal peptide of β - and γ -chains: Tyr-His. There were other peptides which had compositions resembling known peptides from the C-terminal halves of the β - and γ -chains, and

Tryptic peptide residue no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
β -chain			LEU		PRO				SER				ALA			GLY	VAL			ASP		VAL								
ϵ -chain	VAL - HIS - PHE - THR - ALA - GLU - GLU - LYS - ALA - ALA - VAL - THR - SER - LEU - TRP - SER - LYS - MET - ASN - VAL - GLU - ALA - GLY - GLY - GLU - ALA - LEU - GLY - ARG -																													
γ -chain	GLY				GLU		ASP			THR - ILE						GLY	VAL				ASP							THR		
	4										5																			6
	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
	LEU - LEU - VAL - VAL - TYR - PRO - TRP - THR - GLY - ARG - PHE - PHE - ASP - SER - PHE - GLY - ASN - LEU - SER - SER - PRO - SER - ALA - ILE (LEU, GLY, ASN, PRO, LYS) VAL -																													
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
	LYS - ALA - HIS - GLY - LYS - LYS - VAL - LEU - THR - SER - PHE - GLY - ASP - ALA - ILE - LYS - ASN - MET - ASP - ASN - LEU - LYS - PRO (ALA, PHE, ALA, LYS, LEU, SER, GLU, LEU, HIS, CYS, ASP, LYS, LEU, HIS, VAL, ASP, PRO, GLU, ASN, PHE, LYS)																													
	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
	LEU, HIS, CYS, ASP, LYS, LEU, HIS, VAL, ASP, PRO, GLU, ASN, PHE, LYS)																													
	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146				
	GLU, PHE, THR, PRO, GLU, VAL, GLN, ALA, ALA, TRP, GLN, LYS, VAL, ILE, ALA, ALA, VAL, LEU, GLY, ALA, LEU, ALA, SER, LYS) TYR - HIS																													

Fig. 1 Amino acid sequence of the human embryonic ϵ -chain. Residues 1–54, 60–83 and 145–146 are determined sequence, residues 55–59, 84–104 and 111–144 are a tentative sequence constructed by homology with known mammalian β -chains. Only differences in the human β - and γ -chains have been included.

from these a tentative sequence for the second half of the ϵ -chain was constructed. In addition, manual and automatic sequencing of one of the cyanogen bromide fragments and some tryptic peptides derived from it enabled a sequence for residues 41–83 to be established (Fig. 1). All the sequence data corresponded entirely to the amino acid compositions of peptides previously determined. The assignment of amide residues where sequence is not available was made using amino acid composition and electrophoretic mobility of the peptides, as well as homology with other β -like chains.

The sequence of the ϵ -chain established so far shows that it differs from both the β - and γ -chains. Using the definitive sequence information in Fig. 1 there are 24 differences between the ϵ - and β -chains and 18 between ϵ - and γ -chains, compared with 26 differences between β - and γ -chains. Tryptic peptides 4, 6, 7, 8, 11 and 17 maintain the invariant composition found in their equivalents in the human β -, γ - and δ -chains. Further, the ϵ -chain contains four isoleucine residues whereas there are none in the β -chain and three in the γ -chain. There is no cysteine at position 112, explaining the inability to separate the α - from the ϵ -chains after PCMB treatment. The difference in charged amino acids so far established is consistent with the differences in electrophoretic mobility between Hb-Gower 2 and Hb-A or Hb-F.

Hb-Gower 1 could not be isolated using the procedure described above, presumably because it was either not separated from Hb-A or precipitated during the purification. Therefore, as a preliminary step in the isolation of Hb-Gower 1, maternal red cells were separated from the embryonic cells. Although the latter are conspicuously large (~ 15 – $20 \mu\text{m}$ mean cell diameter) and retain their nucleus in a condensed form, they constitute less than 0.01% of the total red cells collected from an abortion sample. Separation of the two cell types was achieved using a large-scale Ørskov-Jacobs-Stewart reaction¹¹ which is based on the low levels of carbonic anhydrase present in fetal cells. The cells were incubated initially for 2 min with acetazolamide and NH_4HCO_3 . Because of the large volumes necessary for the preparation, the mixture was then centrifuged for 5 min at 2,000g at 4 °C to sediment unlysed cells. The latter were resuspended in isotonic saline and spun through Ficoll¹¹. Placental cells and leukocytes were removed by centrifuging the suspension through first 40% and then 60% Percoll at 2,000g at 4 °C

for 5 min. With sufficiently early embryonic samples a preparation containing 100% large nucleated red blood cells could be obtained.

The embryonic red cells were lysed with water and the membranes pelleted by spinning at 18,000g for 15 min. No organic solvents were used as this denatures the haemoglobin. The haemolysates were dialysed against developer A and loaded on to a DEAE-cellulose DE52 column as described previously. After elution of Hb-Gower 2 with developer A a salt gradient was applied by mixing 150 ml developer A containing 0.005 M NaCl in a constant-volume chamber with developer A containing 0.03 M NaCl. Tryptic peptide maps of one haemoglobin fraction which migrated electrophoretically close to Hb-A₂ showed peptides which were characteristic of the ϵ -chain. Of the peptides in Fig. 1, 1, 3, 4, 5, 6, 7, 7–8 and 9 were identified and confirmed by amino acid analyses. Other ninhydrin-positive spots were clearly peptides from the ζ -chain¹², with identical compositions for residues 17–31 (ζ 4), 32–40 (ζ 5), 57–61 (ζ 7–8), 90–92 (ζ 10), 93–99 (ζ 11) and the C-terminal peptide, residues 140–141 (ζ 14).

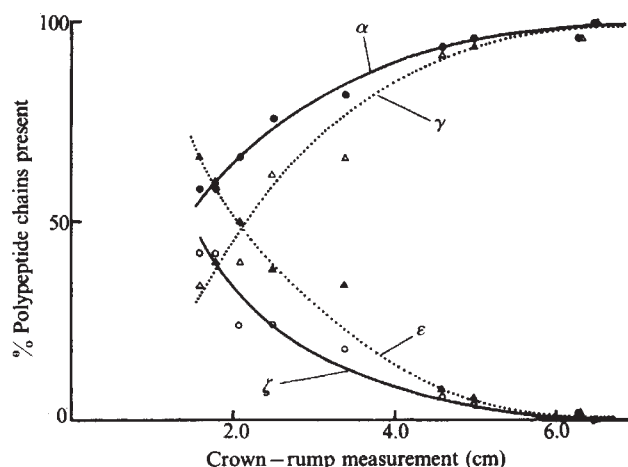


Fig. 2 Developmental changes in human globin chains. The curves have been constructed using the data in refs 1, 2 and 15 and the composition for Hb-Gower 1 $\zeta_2\epsilon_2$.

These results confirm previous suggestions¹³ that the ζ -chain is the human embryonic α -type-chain and that Hb-Gower 1 has the structure $\zeta_2\epsilon_2$. This structure explains the finding that oxygen dissociation studies of embryonic red cells show a full cooperative effect¹⁴. Furthermore, the globin chain composition of the human embryonic haemoglobins corresponds to that found in other mammals. From the available data on the haemoglobins in embryos at various stages of development it can be concluded that the disappearance of the ζ - and ϵ -chains occurs in parallel (Fig. 2), suggesting that there is a coordinated change-over from the production of the embryonic ζ - and ϵ -chains to the fetal α - and γ -chains which occur at about the same time as the liver replaces the yolk sac as the main site of erythropoiesis.

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Note added in proof: Proudfoot and Baralle¹⁶ have recently cloned a human ϵ -globin gene. The DNA sequence of a fragment of this gene gives an amino acid sequence which corresponds exactly with residues 61–100 in Fig. 1.

R. E. GALE
J. B. CLEGG*
E. R. HUEHNS

Department of Clinical Haematology,
University College Hospital Medical School,
London WC1, UK

*MRC Unit for Molecular Haematology,
Nuffield Department of Clinical Medicine,
Radcliffe Infirmary, Oxford, UK

Received 14 March; accepted 23 May 1979.

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K562 human leukaemic cells synthesise embryonic haemoglobin in response to haemin

STUDIES of the regulation of human erythropoiesis and haemoglobin synthesis at different phases of development are hampered by the lack of a self-sustaining culture in which erythroid differentiation can be examined, and by difficulties in obtaining erythropoietic tissue in embryonic and early fetal life¹. New insights into the regulation of murine erythropoiesis have been obtained by the study of established cultures of erythroleukaemic cells (Friend cells) which can be induced to differentiate *in vitro*². Recently, Andersson *et al.*^{3,4} have reported that the K562 human cell line, obtained from a patient with an acute transformation of chronic myeloid leukaemia (CML) has properties in common with erythroid cells. In particular, cells of this

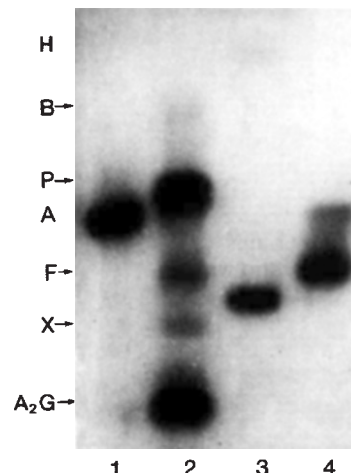


Fig. 1 Cells were grown for 6 days in the presence of 0.1 mM haemin. They were then spun down, washed three times in Hank's balanced salt solution and the final packed cell pellet resuspended in an equal volume of distilled water and lysed by three cycles of freeze/thawing. Stroma was spun out at 2,000 r.p.m. for 10 min and 40,000 r.p.m. for 45 min. Haemoglobins were separated by starch gel electrophoresis at pH 8.6 (ref. 6). Bands were visible with the naked eye and stained for photography with benzidine⁵. Bands in the sample (channel 2) were identified as Hb-Bart's (B), Hb-Portland (P), Hb-F, Hb-X and Hb-Gower 1 (G) by reference to the following standards: channel 1, Hb-A + Hb-A₂; channel 3, Hb-Q and Hb-H; channel 4, Hb-A + Hb-F.

line contain glycophorin and spectrin in their membranes and synthesise minute amounts of haemoglobin, detectable by radioimmunoassay. We have investigated the capacity of these cells to differentiate *in vitro* and report here that they are induced by haemin to synthesise large amounts of haemoglobin; preliminary analysis indicates that this is predominantly of the embryonic type.

Various potential inducers, including dimethyl sulphoxide, *n*-butyric acid, *N*-methylacetamide and hexamethylene-bisacetamide, were tested for their effect on K562 cells and proved ineffective at concentrations that induce Friend cells to synthesise haemoglobin. However, K562 cells grown in the presence of haemin synthesised large amounts of haemoglobin. The cells were subcultured twice weekly in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum. Haemin was added to cultures in logarithmic growth to a concentration 0.1 mM. The presence of haemoglobin was confirmed by absorption bands at 540, 576 and 415 nm and quantitated at 415 nm (ref. 5). Cells cultured with haemin accumulated 3 pg haemoglobin compared with 0.3 pg without haemin. The haemoglobin seemed to be relatively evenly distributed within the cells and we did not observe the benzidine-positive particles reported by Andersson *et al.*⁴. Shedding of cytoplasmic blebs was observed but was rare and was not greater than seen in control cells.

Cells were grown for 6 d with 0.1 mM haemin, lysed with 1 volume of water, the membranes removed by centrifugation and the haemoglobin analysed by starch gel electrophoresis⁶ (Fig. 1). Two major haemoglobin components were found, one migrating slightly faster than a Hb-A marker in the position of the embryonic haemoglobin, Hb-Portland ($\zeta_2\gamma_2$)⁷ and the other slightly more slowly than Hb-A₂ in the position of another embryonic haemoglobin, Hb-Gower 1 ($\zeta_2\epsilon_2$)^{8,9}. In addition, there were trace amounts of fractions moving in the positions of Hb-F ($\alpha_2\gamma_2$) and Hb-Bart's (γ_4), and an as yet unidentified haemoglobin (fraction X) found in variable amounts in early embryos¹⁰. The 'Hb-Gower 1' fraction was isolated from 6-day induced K562 cells by a combination of gel filtration and DEAE-cellulose chromatography. Following digestion with trypsin, fingerprinting and staining with ninhydrin, peptides

were found which corresponded to ζ and ϵ peptides of Hb-Gower 1. The identity of peptides ϵ I and ζ V were confirmed by amino acid analysis.

Further evidence that the induced K562 cells synthesis γ -globin chains and small amounts of α -chains was obtained by incubating the cells with ^3H -leucine, separating the globin chains by CM-cellulose chromatography and determining the amount of radioactivity incorporated into each chain¹¹⁻¹². It was found that γ - and α -chains were labelled and that the α/γ -globin chain synthesis ratio was 0.08; no β -chain synthesis was detected. Presumably, the small numbers of α -chains synthesised in these cells combine mainly with γ -chains to produce Hb-F and the remainder of the γ -chains are associated with ζ -chains in Hb-Portland, the excess forming the γ_4 -tetramers of Hb-Bart's¹. It has previously been shown that ζ and ϵ chains are not recoverable in this chromatographic system^{9,13}.

The reason for the induction of embryonic haemoglobin synthesis in this cell line is not clear. Neither embryonic nor fetal haemoglobins have been found in association with adult CML¹⁴. The electrophoretic pattern of K562 cell haemoglobin corresponds closely with that observed in haemoglobins from purified embryonic erythroblasts⁷⁻¹⁰. The subunit compositions of the human embryonic haemoglobins are now known^{7-9,13,15} although the amino acid sequences of their component chains are still incomplete. The present observations on the haemoglobins of K562 cells suggest that these cells may offer a source of embryonic haemoglobins for the further elucidation of their structure and function, and that they may provide a useful model for studying the developmental biology of human embryonic erythroblasts and for examining the mechanism of the switch from embryonic to fetal haemoglobin synthesis.

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T. R. RUTHERFORD
J. B. CLEGG
D. J. WEATHERALL

MRC Molecular Haematology Unit,
University of Oxford,
Nuffield Department of Clinical Medicine,
The Radcliffe Infirmary, Oxford, UK

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Induction of sporulation in *Bacillus brevis* by peptide antibiotics

THE regulatory mechanisms of the process of bacterial sporulation are unknown, although some authors have speculated that sporulation and the synthesis of peptide antibiotics are somehow connected¹⁻⁴. Paulus and coworkers proposed that tyrocidine and gramicidin D, both synthesised by *Bacillus brevis*, may interact with the transcriptional process during sporogenesis⁵.

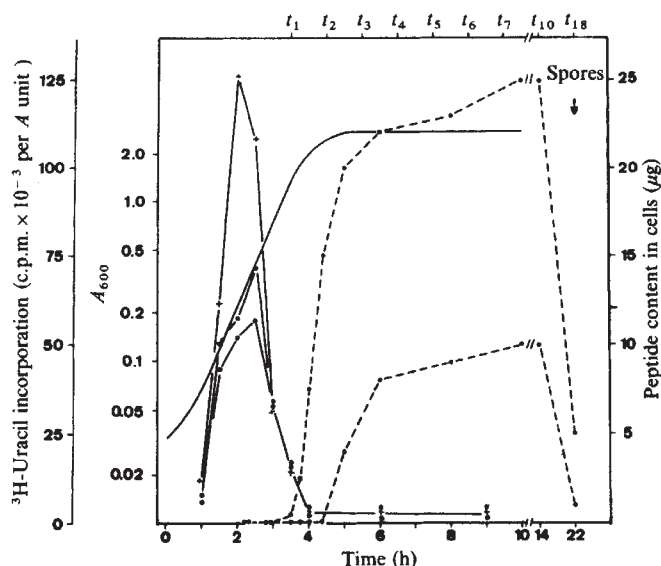


Fig. 1 *Bacillus brevis* (ATCC 8185) cells were cultured in Hanson's medium⁷ at 37°C with aeration in a water bath shaker. Growth was followed as a function of time by measuring the absorbance at 600 nm (—). One A unit corresponds to 7×10^6 cells ml^{-1} . At indicated times 1-ml samples were incubated with 20 μg of tyrocidine (●—●) (Serva), gramicidin D (Serva) (○—○) or without antibiotics (+—+) for 10 min and then supplemented with 0.2 μCi of ^3H -uracil (Amersham Buchler, specific activity 48 Ci mmol^{-1}). After 5 min incubation at 37°C, samples were treated with trichloroacetic acid (final concentration 5%) at 0°C. The acid-precipitable material was collected on glass fibre filters and the radioactivity measured in a scintillation counter. Radioactivity as c.p.m. per A unit was plotted against time of incubation. In parallel, the tyrocidine and gramicidin D content of cells was estimated by Sephadex L-20 column chromatography according to the method of Bartley *et al.*⁸. The tyrocidine (●—●) and gramicidin D content (○—○) is plotted as μg peptide in cells in 1 ml of the culture.

They have isolated a mutant of *B. brevis* which is unable to synthesise gramicidin D and which produces defective spores. The defects could be cured by exogenous gramicidin D, suggesting that this peptide is involved in sporogenesis⁶. In this report we demonstrate that sporulation of early vegetative growing *B. brevis* cells can be induced by exogenous tyrothricin, a mixture of tyrocidine and gramicidin D, provided that the cells are exposed to a culture medium lacking the nitrogen source. The induction of sporulation occurs concomitantly with a significant increase in RNA synthesis. The results suggest that sporulation and the action of these two peptides are causally connected. Both peptides may play a positive regulatory role in cell differentiation.

Figure 1 shows some characteristics of a *B. brevis* culture grown in Hanson's medium⁷. The beginning of spore formation can be detected microscopically at t_4 , which corresponds to stage III of the sporulation as defined for *Bacillus subtilis*². Spores are released at t_{18} . The production of tyrocidine begins at t_0 , and that of gramicidin D between t_1 and t_5 . From t_3 the peptide concentration in the cells increases only slightly. Free spores, however, contain only 20% of the tyrocidine and 10% of the gramicidin D found in sporulating cells. We investigated the RNA synthesis of cells at different times during growth by testing their ability to incorporate tritiated uracil. Figure 1 shows the uracil incorporation into acid-precipitable material within 5 min. The uptake increases up to the early logarithmic growing phase, decreases in the mid and late logarithmic phase and becomes low in sporulating cells. This decrease could reflect a lowered permeability of the cells for uracil. The uracil incorporation was also studied in the presence of 20 $\mu\text{g ml}^{-1}$ of tyrocidine or gramicidin D (Fig. 1). At the beginning of growth the cells were relatively insensitive to

the peptides, but became sensitive in the early logarithmic phase; their RNA synthesis was dose-dependently inhibited by the peptides (not shown). This sensitivity decreased when the cells were in the mid logarithmic phase and, finally, the cells became completely insensitive.

Based on the observation depicted in Fig. 1 we tried to induce sporulation in cells at different stages of growth by the addition of tyrothricin, the natural mixture of tyrocidine and gramicidin D (about 2:1). The addition of tyrothricin to mid and late logarithmic cells had no effect on sporulation: treated and untreated cells started to sporulate within the same time. Addition of tyrothricin to early logarithmic cells inhibited RNA synthesis and at higher concentrations led to cell death.

In another approach cells were grown in Hanson's medium up to 0.2–0.3 A units, at which their uracil incorporation was sensitive to exogenous peptides, and were then transferred to a modified medium defined by Mach⁹ containing glycerol as the carbon source but no nitrogen (C^+N^- medium). On addition of tyrothricin these cells began to sporulate and 5 h later had the microscopical appearance of prespores of stage III. The number of sporulating cells was dependent on the tyrothricin concentration (not shown).

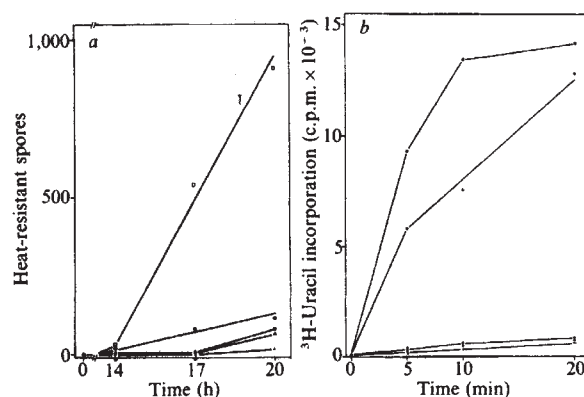


Fig. 2 The effect of tyrothricin on the formation of heat-resistant spores and on RNA synthesis of *B. brevis*. Cells were grown in Hanson's medium to an absorbance of 0.2, then filtered, washed and suspended in C^+N^- medium in the presence of 0 (+—+), 5 (Δ — Δ), 10 ($\square$ — $\square$), 20 ($\bullet$ — $\bullet$) and 40 ($\square$ — $\square$) $\mu\text{g ml}^{-1}$ of tyrothricin. *a*, After incubation for the indicated times at 37 °C, samples were heated for 20 min at 80 °C. Aliquots were plated on agar plates containing the components of Hanson's medium. The number of colonies was plotted against incubation time. The total number of viable cells was estimated by plating as 10^4 . *b*, After 14 h incubation in C^+N^- medium 1-ml samples of the culture were incubated with 0.2 μCi of ³H-uracil (Amersham Buchler, specific activity 48 Ci mmol⁻¹). At the indicated times the uracil uptake was stopped by the addition of trichloroacetic acid and the precipitates then treated as described in Fig. 1. The ³H-uracil incorporation was plotted as c.p.m. against incubation time.

Figure 2a shows the onset of the formation of heat-resistant spores and indicates that at low tyrothricin concentrations only a few spores become heat resistant. At concentrations comparable with that extracted from sporulating cells (Fig. 1) there is a dramatic increase in heat-resistant spores, whereas in the absence of tyrothricin only a few heat-resistant spores are found after 20 h. We conclude from these results that exogenous tyrothricin induces the sporulation process of early vegetative cells. A specific metabolic situation due to the absence of nitrogen might be a prerequisite for the sporulation-inducing activity of the two peptides. In a parallel experiment, cells were incubated in C^+N^- medium in the presence of equivalent amounts of either gramicidin D or tyrocidine. With gramicidin D no prespores were detectable; with tyrocidine less than 10% of the cells formed prespores after 14 h. These results clearly

indicate that the induction of sporulation must result from specific actions of both peptides.

If early vegetative cells incubated in C^+N^- medium in the presence of tyrothricin undergo sporulation they may exhibit rates of RNA synthesis different from those of cells starved in the same medium but in the absence of tyrothricin. This was tested by transferring early vegetative cells into C^+N^- medium with or without tyrothricin. After incubation for 14 h the cells were tested for their RNA synthesis. As shown in Fig. 2b, the rate of uracil incorporation is largely increased by tyrothricin at concentrations necessary for the induction of sporulation (Fig. 2a).

How might tyrocidine and gramicidin D act at the molecular level? Earlier studies indicated that tyrocidine inhibits RNA synthesis *in vivo*^{5,10} and *in vitro*^{5,11}, in the latter by forming a complex with the DNA¹¹ and interacting with the initiation process of transcription¹². Also, gramicidin D inhibited RNA synthesis^{13,14} by destabilising the open initiation complex¹⁵. However, when gramicidin D was added to a tyrocidine-inhibited transcription system reactivation of transcription was observed¹³. This reactivation was only partial and was specifically dependent on the RNA polymerase from *B. brevis* and on a well defined equilibrium between tyrocidine and gramicidin D¹⁶. Studies on the molecular mechanism of this reactivation suggested that gramicidin D interacts with the DNA-tyrocidine complex, thereby weakening the DNA-tyrocidine interaction, and thus allows the RNA polymerase to transcribe^{12,17}. As both peptides affect the transcriptional process they could be involved in gene regulation during differentiation of *B. brevis*. Tyrocidine could act as a nonspecific repressor, turning off transcription, whereas gramicidin D could de-repress part of the genome to allow transcription of those genes necessary for the spore-forming process. Different RNA polymerases¹⁸ or additional protein factors¹⁹ might be essential for the selection of these DNA sequences. Much more work is required to verify this hypothesis. The results presented, however, suggest that the two peptides are, in fact, involved in the control of sporulation.

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HANSJÜRGEN RISTOW
WOLFGANG PSCHORN
JUTTA HANSEN
UTE WINKEL

Institut für Genetik,
Freie Universität Berlin, Arnimallee 5–7,
D-1000 Berlin 33, FRG

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Bacillus megaterium resistance to cloxacillin accompanied by a compensatory change in penicillin binding proteins

BACTERIA have been shown to become resistant to β -Lactam antibiotics (penicillin and cephalosporins) by the following methods: decreased permeability^{1,2}, increased production of an enzyme (β -lactamase) which degrades the antibiotics¹, acquisition of a plasmid that produces a β -lactamase^{3,4}, and a decreased affinity of the lethal target for the antibiotic⁵. β -Lactam antibiotics are thought to kill bacteria by interfering with the terminal stages of cell wall biosynthesis⁶. The cross-linking of the cell wall by a transpeptidase enzyme has been implicated as the probable target site^{7,8}, although inhibition of a carboxypeptidase⁹ and stimulation of autolytic activity¹⁰ have also been proposed. It has been suggested that penicillin is a transition state analogue of the D-alanyl-D-alanine moiety of the donor molecule in the transpeptidation reaction¹¹ and that penicillin binds covalently to the transpeptidase⁷. Investigations therefore concentrated on enzymes which could fix penicillin in bacterial membranes^{12,13}. Bacteria contain multiple penicillin binding proteins (PBPs) and it is important to identify which one is the lethal target. One approach used in this laboratory has been to purify the individual PBPs and investigate their enzymatic activity, although it was realised that solubilisation might lead to inactivation. In *Bacillus megaterium* the only PBP shown to possess enzymatic activity was PBP 5 (ref. 14) (the one of lowest molecular weight), and this was the D,D-Ala carboxypeptidase, which is much less sensitive to β -lactams than is growth of the organism¹⁵. Despite the lack of enzymatic activity the affinity of the remaining PBPs 1–4 for benzylpenicillin could be measured in whole cells. PBP 1 was the only protein that interacted covalently with benzylpenicillin at the lowest concentration of the antibiotic that would inhibit growth, and it was therefore proposed to be the lethal target¹⁶. An alternative approach is to determine which PBPs become altered as an organism develops resistance to an antibiotic. This method has shown that resistance can be due to a

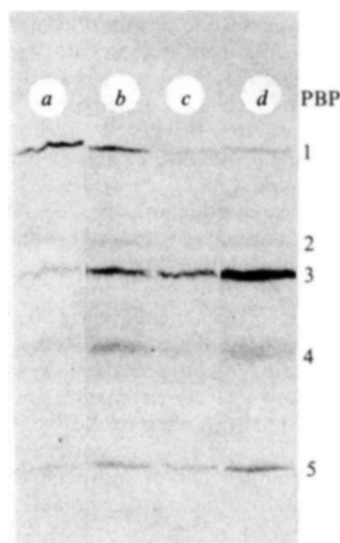


Fig. 1 Penicillin binding proteins (PBPs) of the parent and cloxacillin-resistant mutants of *B. megaterium*. The PBPs were examined as described in ref. 14. Protoplast membranes were prepared from exponentially growing cells and treated with ¹⁴C-benzylpenicillin (10 μ g ml⁻¹) for 10 min at 37 °C. The proteins were then fractionated by SDS-polyacrylamide slab-gel electrophoresis and the PBPs detected by fluorography: a, parent; b, XR2r; c, XR2; d, XR3.

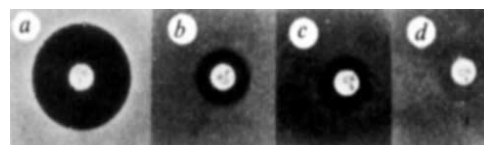


Fig. 2 Cloxacillin sensitivity of the parent and mutant organisms. Bacteria were grown in Penassay broth to a density of 0.4 mg dry wt per ml; 0.2 ml cells were added to 20 ml Penassay agar at 46 °C and overlaid onto a 9-cm plate containing 30 ml Penassay agar. Cloxacillin sensitivity disks (Mast) were applied to the centre of each plate and diffusion allowed to occur at 4 °C for 2 h. Plates were incubated overnight at 37 °C: a, parent; b, XR2r; c, XR2; d, XR3.

PBP having a decreased affinity for a β -lactam antibiotic. Using this technique PBP 2 has been identified as the lethal target for mecillinam in *Escherichia coli*⁵ and for cloxacillin in *Bacillus subtilis*¹⁷. However, in this report a new mode of resistance is described in which there is a purely quantitative change in the ratio of PBP 1 to PBP 3 as resistance to cloxacillin develops in *B. megaterium*. Thus, resistance results from a decrease in the level of the lethal target which is compensated by an increase in a less sensitive target.

Spontaneously resistant bacteria were isolated in a stepwise fashion¹⁷. *Bacillus megaterium* was grown on plates of Penassay agar containing cloxacillin at 37 °C. Cloxacillin was chosen to avoid selection of β -lactamase-producing organisms as it is not degraded by the β -lactamase from *B. megaterium*. A resistant mutant, designated XR2, was then purified by three rounds of single colony isolation. XR2 was filamentous and grew more slowly (mean generation time 40 min) than the rod-shaped parent (mean generation time 25 min). Membranes were prepared from XR2, treated with ¹⁴C-benzylpenicillin, the proteins fractionated by SDS-polyacrylamide slab-gel electrophoresis and the PBPs detected by fluorography^{18,19}. The results indicated an increase in the amount of PBP 3 and a decrease in PBP 1 (Fig. 1c) compared with the parent (Fig. 1a).

If the ratio of PBP 3 to PBP 1 is important in the development of cloxacillin resistance, one would expect a further increase in this ratio if the PBPs are examined in a mutant with an increased resistance to cloxacillin. Such a mutant (XR3) was derived from XR2, and was again filamentous and grew more slowly (mean generation time 60 min) than XR2. The PBPs of mutant XR3 were examined and the ratio of PBP 3 to PBP 1 had increased (Fig. 1d) relative to XR2. The fact that the cloxacillin-resistant mutants grew more slowly than the wild type suggests that there would be a selective pressure to revert to the wild type when the resistant mutants were grown in the absence of cloxacillin. In an attempt to obtain such revertants mutant XR2 was grown overnight in glucose-limited batch cultures until a morphological change was detected (eight successive subcultures at 24-h intervals). This change involved a more obvious and frequent septation of the filaments. An organism was purified through two rounds of single colony isolation on solid media and designated XR2r (a partial revertant). When the PBPs of XR2r were examined the ratio of PBP 3 to PBP 1 was found to have decreased (Fig. 1b) relative to XR2. The growth rate of XR2r had increased (mean generation time 35 min) and the cloxacillin resistance had decreased relative to XR2.

Thus, we have a series of three mutants in which increasing resistance to cloxacillin (Fig. 2) is related to an increase in the ratio of PBP 3 to PBP 1 (Fig. 1), a decreasing growth rate and filamentation of the organism. To ascertain whether any change in the affinity of the PBPs for cloxacillin had a role in resistance, the sensitivity of the five PBPs was measured for all the mutants (see Fig. 3 for results with the parent and XR3). No change in the affinity of PBPs for cloxacillin in the parent and mutants was detected. However, a difference was observed in the sensitivity profiles of PBP 1 and PBP 3. Cloxacillin bound to PBP 1 of all strains at low concentration (without saturating the protein completely) but required a high concentration (>100 μ g ml⁻¹)

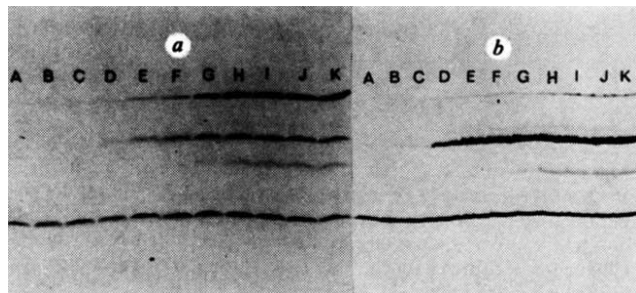


Fig. 3 Sensitivity of penicillin binding proteins (PBPs) to cloxacillin in the parent (a) and XR3 (b). As radioactive cloxacillin was not available the sensitivity of PBPs to cloxacillin was measured by prelabelling the PBPs with unlabelled cloxacillin followed by treatment of the membranes with an excess of ^{14}C -benzylpenicillin which will bind to PBPs unoccupied by cloxacillin (as described for cephalosporins in ref. 14). Experimental details were as for Fig. 1 except that membranes were incubated with cloxacillin for 10 min at 37°C before addition of ^{14}C -benzylpenicillin. The concentrations of cloxacillin were ($\mu\text{g ml}^{-1}$): (A) 100, (B) 30, (C) 10, (D) 3.0, (E) 1.0, (F) 0.3, (G) 0.1, (H) 0.03, (I) 0.01, (J) 0.003, (K) 0.001.

to inhibit completely the subsequent binding of ^{14}C -benzylpenicillin. In contrast, PBP 3 was saturated by cloxacillin at approximately $30 \mu\text{g ml}^{-1}$ but did not apparently interact with the antibiotic at concentrations lower than $3 \mu\text{g ml}^{-1}$ (Fig. 3). It is the sensitivity to low concentrations of cloxacillin which is important as the minimum growth inhibitory concentration for the parent is $0.08 \mu\text{g ml}^{-1}$ (ref. 15).

If the role of PBP 1 in cellular growth can be taken over by PBP 3 then the organism would become more resistant to cloxacillin if PBP 3 was less sensitive to the antibiotic than PBP 1. This situation is apparently fulfilled in the cloxacillin-resistant mutants reported here. There is a 1.5-fold increase in PBP 3 and a 10-fold decrease in PBP 1 in mutant XR2 compared with the parent strain (calculated by standardisation with PBP 5 which is assumed to be unaltered in quantity in all the organisms as it can be ruled out as the lethal target^{14,15}). This may well be sufficient to alter the cloxacillin sensitivity of the organism. Compensatory changes in PBPs have also been observed in *E. coli* where supersensitive mutants lacking PBP 1Bs partially reverted to normal sensitivity to β -lactam antibiotics and this change was accompanied by an increase in PBP 1A and PBP 2 (ref. 20). The results also indicate that PBP 1 is the lethal target for cloxacillin in the wild-type *B. megaterium* and this agrees with the results obtained by a different method with benzylpenicillin¹⁶.

ALLEN F. GILES
PETER E. REYNOLDS

Sub-department of Chemical Microbiology,
Department of Biochemistry,
University of Cambridge,
Tennis Court Road, Cambridge, UK

Received 3 May; accepted 29 May 1979.

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Influence of leaves on sporophore production by fungi forming sheathing mycorrhizas with *Betula* spp.

MOST of the intimate and often beneficial associations formed between plant roots and some soil fungi can be classified as either endomycorrhizas of various types or sheathing (ecto-) mycorrhizas^{1,2}. The latter, in which colonised roots are shorter and thicker than their uncolonised counterparts, are mainly attributed to basidiomycetous fungi that produce sporophores (toadstools) typical of the Agaricales. Sometimes fungi forming sheathing mycorrhizas produce large numbers of sporophores. P. Larsen, quoted by Romell³, estimated sporophore production by the mycorrhizal *Suillus bovinus* (Fries) O. Kuntze to be about 180 kg (dry weight) per hectare per yr. Although their evidence is questionable, experiments done by Romell⁴ and Laiho⁵ suggest that the mycorrhizal *Boletus subtomentosus* Fries and *Paxillus involutus* (Batsch ex Fr.) Fr. usually form sporophores only if they are organically linked to living trees. Hacskeylo⁶ found that shading foliage of *Pinus virginiana* Mill decreased sporophore formation by *Thelephora terrestris* Fr. The results reported here extend the understanding of this relation by showing that sporophore production by some mycorrhizal associates of birch (*Betula* spp.) is strongly dependent, in the field, on the presence of green foliage.

Two experiments, done at the Bush Estate (lat. $55^\circ 52' \text{N}$) 10 km south of Edinburgh, are described here. In one, sporophore production was related to the different rates of natural autumnal leaf yellowing found in saplings grown from seeds of *Betula pendula* collected by Roth from three widely spaced locations, whereas in the other, the effects of experimental defoliation were investigated.

Seeds of *B. pendula*, collected from natural stands at lat. $50^\circ 40' \text{N}$ (southern England), $57^\circ 20' \text{N}$ (Latvia) and $66^\circ 30' \text{N}$ (northern Sweden), were treated with Captan, a 75% fungicidal dust of *N*-trichloro-methyl-mercapto-4-cyclohexene-1,2-dicarboximide, before being sown on 7 January 1971, germinated and subsequently transplanted to pots (approximately 100-mm diameter) of partially sterilised John Innes type compost. After being grown in a glasshouse for 10 months and then hardened-off, three replicate saplings of each seedlot (provenance) were planted in November 1971. They were spaced 3 m apart, within and between rows, in three randomized blocks on an adequately drained medium silty loam soil where their roots were colonised by naturally occurring mycorrhizal fungi. During each succeeding autumn and at intervals of about 2 weeks the position of every sporophore was recorded for each tree. At the same time a note was made of 'percentage leaf yellowing', an integrated measure of yellowing and defoliation.

In 1975, 4 yr after planting, it was apparent that the origin of the *B. pendula* seeds (provenance) greatly affected the production of sporophores, mostly basidiomycetous, but also a few of the ascomycete *Peziza badia*, Persoon ex M  rat, a pattern confirmed in 1976 and 1977. Whereas 81, 150 and 760 sporophores were associated with each tree of southern origin (lat. $50^\circ 40' \text{N}$) in 1975, 1976 and 1977, respectively, only 29, 53 and 370 were found around trees of more northerly origin (lat. $57^\circ 20' \text{N}$), with virtually none, zero, zero and three, associated with those from the most northerly location (lat. $66^\circ 30' \text{N}$) (Fig. 1). In Autumn 1977 the number of species of sheathing mycorrhizal fungi producing sporophores increased from three associated with the 'Swedish' trees (*Hebeloma crustuliniforme* (Bull. ex Saint-Amans) Qu  let, *Hebeloma* sp. and *Inocybe lanuginella* (Schroet.) Lange) to 10 with the southern England provenance which had many specimens of the types already mentioned and also *Cortinarius* sp., *H. fragilipes* Romagn  si, *H. mesophaeum* (Pers. ex Fr.) Qu  let, *H. sacchariolens* Qu  let, *Laccaria laccata* (Scop. ex Fr.) Cooke, *L. proxima* (Boud.) Pat.,

Lactarius pubescens (Fr. ex Krombh.) Fr. and *Leccinum scabrum* (Fries) S. F. Gray.

Total numbers of sporophores, irrespective of species, were inversely related to rates of foliar yellowing. Whereas virtually none was associated with trees grown from seed from lat. 66°30' N, which were completely defoliated in mid-August, many occurred where yellowing was delayed until late September (57°20' N) and October (50°40' N) (Fig. 1).

Because numbers of sporophores associated with *B. pendula* grown from seeds from different latitudes were systematically and inversely related to the rates of natural foliar yellowing, the effects of experimental defoliation (with scissors) were tested during 1978 on 192 saplings of *B. pendula* and *B. pubescens* Ehrh., which were aseptically and vegetatively propagated and planted in 1975. They were arranged in eight randomised blocks each testing 24 (2 × 3 × 4) factorially arranged treatments including complete defoliation on either 25/26 July or 22/23 August compared with controls subject to natural defoliation which started at the end of September and reached 39% and 86% on 5 October and 3 November, respectively.

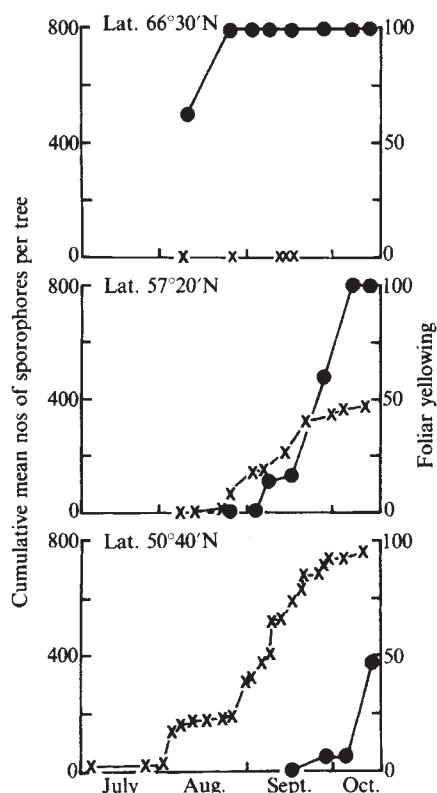


Fig. 1 Patterns during 1977 (6 yr after planting) of seasonal leaf yellowing (●), and the occurrence of sporophores (x) of sheathing mycorrhizal fungi associated with *Betula pendula*, when saplings from seed collected at three different latitudes were grown at lat. 55°52' N (Bush Estate, near Edinburgh).

During 1978 sporophores, which appeared unusually early, were recorded at least twice during each of the six observation periods (A–F) from July to November (Fig. 2). By the end of July sporophores were associated with 25 of the 64 replicate control trees. A peak of 56 was reached in September/October before sporophore production ceased in November. From July to October numbers of newly produced sporophores ranged from 7.8 to 12.6 per tree in the different 3–4 week periods of observation (Fig. 2), contributing to a seasonal total, based on geometric means, of about 45.

During July and early August sporophore production around trees to be defoliated on 22/23 August was similar to that in the control series. As observations made on 25 August indicated, sporophore production ceased immediately after defoliation, with only one tree producing two sporophores during September, contrasting with 55 control trees averaging 10 each. This result duplicates the effects of defoliation in July but, in this instance, axillary and terminal buds started to 'break' in late August with a resultant new flush of leaves. From two trees producing a total of three sporophores in August, numbers of trees with sporophores increased to 16 and 43 in September and October as amounts of foliage increased; this trend was paralleled by increased mean numbers of sporophores per tree which reached 3.8 during October.

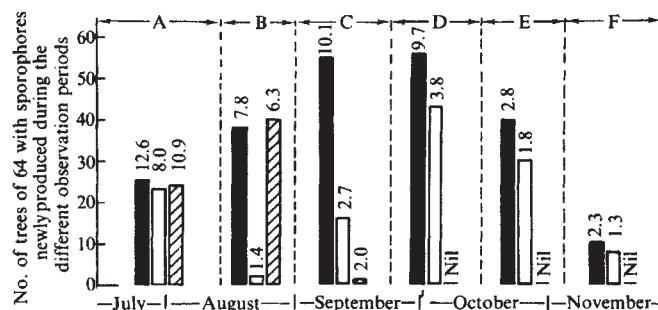


Fig. 2 Mean effects of defoliating *Betula* spp. on either 25 July (□) or 22 August 1978 (▨) on numbers of replicate trees with sporophores of associated mycorrhizal fungi newly produced within the different periods of observation. ■, Naturally defoliating set of control saplings. (Figures at tops of columns are mean numbers of sporophores per tree produced during each period; they were calculated from replicates producing at least one new sporophore during the relevant period.)

The effects of defoliation confirm that the production of sporophores by sheathing mycorrhizal fungi is disrupted when leaf function is impaired. Our observations support Lewis' contention⁷ that many mycorrhizal fungi are 'ecologically obligately symbiotic biotrophs'. But, although they may be entirely dependent on their hosts for carbon⁸, which they sequester, the rapidity of the responses, within 2 d of defoliation, suggest that the involvement of other substances produced by leaves, such as auxins, which can hasten the enlargement of sporophore primordia⁹, should not be overlooked.

F. T. LAST

J. PELHAM

P. A. MASON

K. INGLEBY

*Institute of Terrestrial Ecology,
Bush Estate,
Penicuik,
Midlothian UK*

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matters arising

Upper limits on production rate of NO per ion pair

RECENTLY, Fabian *et al.*¹ have suggested that the rate of production of NO per ion pair during a solar proton event should be 2–2.5 instead of the 1–1.5 used by other authors. They justify the larger rate because of rocket measurements of Arnold² taken during an auroral precipitation event. While Arnold's² measurements may be correct, we question the application of these rates to energetic solar protons as these particles mainly deposit their energy below 80 km. Here we place realistic upper bounds on the NO per ion pair production rate.

First, at altitudes between 80 and 120 km, each N or N⁺ produced can potentially form one NO molecule and each N₂⁺ can potentially form two NO molecules by well known ion chemistry^{3–5}. Thus, by bounding the N, N⁺ and N₂⁺ production rates, we bound the NO production rate.

To calculate the maximum NO production rate, we follow the method of Porter *et al.*⁶. However, in this computation we assume that 20% of the N₂(a¹Π_g) excitations form two NO molecules and that all excitations of N₂ and N₂⁺ with thresholds above 9.76 eV, the dissociation energy, also form two NO molecules. This calculation yields an upper limit of 2.68 NO per ion pair for 10 keV electrons, which is larger and thus consistent with rates implied by the measurements of Arnold² as quoted by Fabian *et al.*¹. Although there are some uncertainties in our calculations due to the cross-sections, we feel that our results are accurate to ±15%, in view of the use of self-consistent energy degradation methods by Porter *et al.*⁶.

Second, we note that at altitudes below 80 km the maximum rate of NO production should be bounded by the production rate of N and N⁺ formation because at these altitudes N₂⁺ reacts almost exclusively to form water clusters and is removed³. In a second calculation we subtract the N₂⁺-produced NO, leaving only the direct N and N⁺ sources of NO and find a maximum production rate of 1.46 and 1.53 NO per ion pair for protons with energies of 20 and 200 MeV, respectively.

We thus conclude that in the altitude

range below 80 km it is difficult to justify NO/ion pair rates greater than about 1.5. In fact, the NO per ion pair production rates for altitudes <80 km are probably close to the 1.2–1.3 calculated independently by Frederick³ and Porter *et al.*⁶. However, in the thermosphere between 80 and 120 km, numbers as high as those quoted by Arnold² are clearly possible.

C. H. JACKMAN*
H. S. PORTER†
J. E. FREDERICK*

* Goddard Space Flight Center,
Laboratory for Planetary Atmospheres,
Greenbelt, Maryland 20771

† Computer Sciences Corporation,
Silver Spring, Maryland 20910

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Amount is not supply rate in energy intake control

ON the theory that rate of energy supply determines intake¹, it is not as Rothwell and Stock state, "logical to assume that isoenergetic stomach loads will have similar effects on voluntary energy intake" (ref. 2), or that—when energy intake does not compensate for changes in energy expenditure—the animals may be "regulating food intake to meet their requirements for some dietary component other than energy" (ref. 3). The amount of energy in the stomach (or anywhere else) does not necessarily have a one-to-one relationship to the instantaneous delivery rate of that energy. The relationship depends on the physiological characteristics of the energy store's turnover.

We have emphasised^{4–7} the relevance of others' findings that the rate of passage of energy from the stomach (and onwards to the tissues) depends to a considerable extent on the form that the energy is in, for example, the energy density of the diet⁸. We have calculated the long-term effects

of some dissociations between amount and supply rate^{5,6} and in the same context pointed out further such mechanistic as well as thermodynamic calculations which are required by an unmodified energy-rate or power-measuring theory of energy intake control. The report by Rothwell and Stock² clearly indicates the importance for theory and practice of extending the simulation work with experimental work to determine the as yet poorly analysed short- and long-term implications of satiation by energy metabolism.

D. A. BOOTH

Department of Psychology,
University of Birmingham,
Birmingham, UK

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ROTHWELL AND STOCK REPLY—If, as Booth claims, "the rate of energy supply determines intake", why do rats force-fed a balanced diet adjust voluntary intake such that their total energy intake is the same¹ as that of free-feeding controls? The rate of energy supply is radically different in these two situations and should therefore produce marked differences in intake; it certainly causes differences in energy retention because the force-fed rats become obese. This latter finding indicates that a greater fraction of absorbed energy is available to metabolism and should, also according to Booth², result in compensatory adjustments to energy intake. In our original paper¹ we suggested that the theory of intake control proposed by Booth and Toates^{3,4} was unlikely to provide an explanation of our results. Given that neither the rate of energy supply nor the metabolic fate of that supply influences intake, we see no reason for modifying that statement and, as a comment on Booth's final sentence, suggest that it is

obviously necessary to extend the 'simulation work' if it is to keep pace with experimental developments.

N. J. ROTHWELL
M. J. STOCK

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ROTHWELL AND STOCK¹ demonstrated that partially tube-fed rats gained significantly more weight than their free-feeding controls even though both groups ate very nearly the same amount. We suggest the possibility that the weight differences result from changed levels of activity. This approach obviates the paradoxical problems suggested by Rothwell and Stock.

A single energy reservoir model is used, as the rats are mature adult males unlikely to change their fat-free weight on over-feeding. We can describe the rate of change of fat² quantities by the following differential equation

$$\alpha \, df/dt = \epsilon P - \text{BMR} - \delta(l + f) \quad (1)$$

where α is the energy equivalent of fat, f , t is time, ϵ is the efficiency of food utilisation, P is the average daily food energy intake, BMR is the basal metabolic rate, l is the fat-free weight assumed to be constant or slowly varying, and δ is a constant independent of the total weight and indicates the average activity level of the rats. We have made no direct distinction between feeding and non-feeding activity of the rats. MacMillan *et al.*³ have demonstrated densitometrically that in man the BMR is independent of the amount of fat present; we assume this to be valid in the case of rats also. Because the BMR is independent of f we can solve equation (1) directly obtaining

$$w = [(\epsilon P - \text{BMR})/\delta][1 - \exp(-\delta t/\alpha)] + w_0 \exp(-\delta t/\alpha) \quad (2)$$

where we have replaced $(l + f)$ by the total body weight w and $(l + f_0)$ by the initial total body weight w_0 .

We used Kleiber's 3/4-power law⁴ to deduce the BMR. The BMR of a 'standard' 65-kg man is 6.27 MJ per day (1,500 kcal d⁻¹)⁵; thus, the average rat will

have a BMR of 128 kJ per day. Within the rat groupings we scale the BMR by a 2/3-power law as Kleiber's rule only relates to average interspecies quantities.

To use equation (2) we take α to be 39.2 MJ per kg (9,370 kcal per kg)⁵. The value of ϵ is unknown but should reflect losses due to the thermic effect and the passing of small energy amounts in the faeces. Antonetti⁶ used a value of 0.9 in man. We take ϵ to be unity, because this must be the limiting case, and apply it to both tube-fed and free-feeding rats. Table 1 shows the data of Rothwell and Stock¹ and includes the results of our calculations. There is enough information to solve equation (2) for the activity coefficient, δ (Table 1).

In each case δ was less for the tube-fed rats than for the control groups, suggesting that the observed weight gain difference can be explained by the decreased activity of the test animals. The possibility that the tube-fed rats might increase their non-feeding activity is not reflected in the decreased values of δ . The average value of the activity coefficient of the controls was (423 ± 73) kJ per kg per day; the daily activity energy expenditure was 153 ± 26 kJ.

We calculate that the average control rat expended about 54% of the total energy on all forms of activity. Rothwell and Stock¹ consider activity energy to be negligible; Miller and Mumford⁷ deduce that about 3% of the total energy is expended on activity by severely exercised rats by applying a fixed energy expenditure factor of 0.5 kcal per kg per km to rat, man and elephant. Morrison⁸ has measured spontaneous activity calorimetrically and reports it to be 25% of the total energy expenditure. Our results agree slightly better with those of Morrison⁸ by assuming ϵ to be 0.9 (ref. 6). Repeating the numerical calculation in this case we find δ to be 333 ± 56 kJ per kg per day, giving a 48% value for activity.

We can deduce the level of activity for rats completely tube-fed. We have plotted in Fig. 1 the ratio of the activity coefficient of the tube-fed rats to that of the control group as a function of the fraction of tube-fed energy. We have included the limiting point for no tube-feeding. The linear fit extrapolated to the limit of complete tube-feeding gives a value of 0.25. We conclude that 75% of caged rat

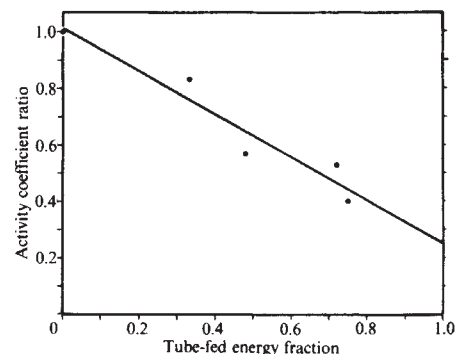


Fig. 1 The ratio of the activity coefficient of the tube-fed rats to that of the appropriate control group plotted as a function of the fraction of tube-fed energy. The point on the ordinate is the limiting value of the ratio for no tube-feeding. The other points are experimental. The linear least-squares fit is displayed and interpolates to a value of 0.25 for complete tube-feeding.

activity is food related. This value cannot be compared with Morrison's results⁸ because our definition of food-related activity is broader.

Equation (2) essentially describes the exponential increase of fat and allows an interesting speculation. The equilibrium value is approached to within $1/e$ of its final value in a time given by α/δ which is 93 ± 16 days. If a parameter proportional to the fat store size is sensed as an 'error signal' for a regenerative control process, and if the sensing time is similar to the rise time of the fat store, we can conclude that experimental studies dealing with problems of control must be carried out for times of the order of or longer than 93 days.

SEYMOUR S. ALPERT

Department of Physics and Astronomy,
The University of New Mexico,
Albuquerque, New Mexico 87131

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Table 1 Data and calculated quantities

Expt	No. of animals	Av. initial weight, w_0 (g)	Av. final weight, w (g)	Energy intake, P (kJ d ⁻¹)	Duration of expt (d)	Fraction of tube-fed energy (%)	BMR, calculated (kJ d ⁻¹)	Activity coefficient, δ , calculated (kJ kg ⁻¹ d ⁻¹)
1 Control	6	436	480	378	30		145	383
Tube-fed	5	440	511	388	30	33.4	146	314
2 Control	12	321	388	405	21		118	454
Tube-fed	8	320	420	400	21	48.1	118	258
3 Control	6	300	358	385	23		113	525
Tube-fed	6	290	385	366	23	71.8	110	278
4 Control	12	402	442	367	21		137	367
Tube-fed	9	400	489	367	21	74.7	136	147

ROTHWELL AND STOCK REPLY—We need not go beyond Alpert's first paragraph to see that the paradox in the control of energy intake revealed in our paper and further discussed below (see reply to Mrosovsky) has been confused with the question of why tube-fed rats gain more weight than control rats on identical energy intakes.

Our paper was not intended to explain this effect of 'meal-feeding' on energy metabolism; we simply took advantage of this well known effect so as to induce excessive weight gains and observe the effect on residual voluntary intake. We were concerned with how intake is related to the regulation of energy balance whereas Alpert is concerned with mechanisms responsible for the greater efficiency of energy utilisation in tube-fed rats.

Although Alpert's theoretical considerations are not pertinent to the paradox discussed in our paper we would, nevertheless, like to make the following points.

First, differences in body energy gain in animals on identical metabolisable energy intakes must be due to differences in energy expenditure. Apart from the 'basal' contribution, expenditure can vary due to changes in activity and/or the efficiency of energetic transformations within the body (that is, diet-induced thermogenesis or the heat increment of feeding). Alpert assumes that the efficiency of energy utilisation within the body is constant and therefore ascribes differences in total energy expenditure entirely to changes in activity. He states that this is logical and mathematically consistent but ignores the fact that it is just as logical and mathematically correct to fix activity at a constant level and ascribe the changes in total energy expenditure to differences in the efficiency of utilisation.

Second, there is evidence that metabolic efficiency is greater in meal-fed animals (see ref. 1 for review). Activity may also be affected but observations have not revealed any noticeable differences. Furthermore, we have calculated the difference in activity that would be required to explain the greater energy retention of our tube-fed rats. Parkes² has determined the energy cost of walking in the rat to be 0.105 kJ per km per g body weight. Applying this value to the rats which were tube-fed 74% of control intake, we estimate that control rats would have to walk 2,000 m per day more than tube-fed rats to explain the differences in energy retention. Note that these were adult rats housed in pairs in cages with floor dimensions of 40 cm × 30 cm, so that it is extremely unlikely, if not physically impossible, for two rats to each walk 2 km per day in these conditions—and even this assumes that tube-fed rats were completely inactive.

In conclusion, we are not convinced by Alpert's deductions concerning the cause of the greater energy retention in our

tube-fed rats and he has not attempted to explain why these rats failed to depress voluntary intake to compensate for their excessive weight gain.

N. J. ROTHWELL
M. J. STOCK

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Rothwell and Stock's paradox and multiple controls of feeding

ROTHWELL AND STOCK¹ reported recently that rats tube-fed a balanced diet intragastrically ingest overall (the tube-fed plus the voluntary intake) the same amount of energy as do control rats. However, the tube-fed rats gained more weight, presumably because of increased efficiency of energy utilisation associated with receiving intragastric loads in a few discrete meals. They considered it paradoxical that the total energy intake was not affected by the fate of the ingested energy.

These are interesting results, but how paradoxical they appear may depend on how strongly one considers energy content of the body to be the fundamental determinant of food intake. Most people, however, would probably accept that intake is under multiple controls, including such factors as palatability, previous conditioning and cost in obtaining food. Energy content of the body could be one factor in the control of food intake but is not necessarily the most important factor in every instance².

To determine if energy content of the body is one factor that affects food intake, or utilisation, in the intragastric tube-feeding situation, further analyses of the data might be useful. For instance, intake during the last few days of Rothwell and Stock's experiment, when the tube-fed rats were somewhat heavier than controls, could be compared with that during the first few days when the animals were similar in weight; if there were differences, energy content of the body would be implicated. If, on the other hand, it could be shown that intake and utilisation in the intragastric tube-feeding situation are independent of body weight, this would be much more surprising than the fact that tube-fed animals fail to control their intake in a way that maintains constant body weight. However, to show conclusively that intake is independent of weight might require longer experiments involving greater weight gains than those occurring in Rothwell and Stock's study.

N. MROSOVSKY

*Departments of Psychology and Zoology,
University of Toronto,
Toronto, Ontario M5S 1A1, Canada*

1. Rothwell, N. J. & Stock, M. J. *Nature* **273**, 146–147 (1978).
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ROTHWELL AND STOCK REPLY—Mrosovsky suggests further analyses of the data to see if intake and utilisation are independent of the changes in body weight occurring during the experiment. A comparison of the energy intake of tube-fed rats during the first 5 days of each experiment with the intake during the last 5 days shows that they are identical, in spite of weight gains equivalent to 20% of starting weight. Furthermore, the rate of weight gain was remarkably constant throughout each experiment, therefore suggesting that both intake and utilisation were independent of body weight. By his own admission, Mrosovsky will be surprised by these results but we would agree that a conclusive demonstration of this independence of intake and body weight probably requires longer experiments with greater weight gains.

N. J. ROTHWELL
M. J. STOCK

*Department of Physiology,
Queen Elizabeth College,
University of London,
Campden Hill Road,
London W8, UK*

Is the benzodiazepine receptor coupled to a chloride anion channel?

FROM their observations on the ability of several anions to facilitate the binding of ³H-diazepam to membrane fragments from rat cortical tissue, Costa, Rodbard and Pert¹ have inferred that this binding site is closely associated with a chloride ion channel. Their data are qualitatively similar to ours with regard to the rank order of potency of the anions and we can confirm that the bromide ion also causes a significant increase in the affinity of the benzodiazepine receptor for the ligand diazepam without significantly affecting the total number of sites available.

Costa *et al.*¹ have drawn a correlation between the ability of various anions to enhance ³H-diazepam binding and their ability to penetrate the activated inhibitory postsynaptic membrane of cat motoneurons as reported by Eccles². As the binding experiments were carried out in cortical tissue membrane fragments it is probably advisable to attempt correlations with electrophysiological data on chloride channels obtained in the same region of the central nervous system. Such data are available in the literature³ and comparison of the electrophysiological data from Eccles² and Kelly *et al.*³ immediately shows that the anion-size specificity in cortical tissue is considerably less than that in the spinal cord; this may

be explained by the fact that the chloride channel in the spinal cord is probably glycine linked whereas that in the cortex is linked to γ -aminobutyric acid.

We wish to refine the bulk correlations attempted by Costa *et al.*¹ by ranking the ability of the various anions to facilitate specific ^3H -diazepam binding and attempting correlations between the rank order so obtained and the ability of these ions to reverse the cortical inhibitory postsynaptic potential (i.p.s.p.) as reported by Kelly *et al.*³. It is difficult to ascertain which parameter from the anion facilitation data should be selected for such a correlation: percentage increase of ^3H -diazepam binding at maximal facilitation, percentage increase at an arbitrarily selected ion concentration, or the lowest ion concentration at which facilitation is seen. One parameter which encompasses these various possibilities is obtained by simply measuring the area under the curves for the facilitation of specific ^3H -diazepam binding against ion concentration (0–200 nM) (Table 1). None of the above correlations reached statistical significance, in the latter case the correlation coefficient with the area under the curve and the percentage probability of reversal of the i.p.s.p. was 0.61 whereas with the mean time to equilibrium it was 0.28. Using the non-parametric Spearman rank test we again found no significant correlation. The major criticism which can be raised against the use of these electrophysiological data is that the technical complexity of such experiments leads to quite large standard errors. It is interesting that Araki *et al.*⁴ also observed differences in the ability of various anions to penetrate the cell membrane and in this case also we have been unable to find a significant correlation with the ability of the ions to facilitate ^3H -diazepam binding.

An alternative explanation is that the effects of these anions are due to their chaotropic properties. This possibility is not favoured by Costa *et al.*¹ because they feel that the concentration ranges over which chaotropic properties are normally observed are generally higher than the ED_{50} values for the anions in facilitating ^3H -diazepam binding, and also that perchlorate, while exhibiting powerful chaotropic properties, is relatively inactive in facilitating binding. In our study F^- caused a significant though small facilitation of the binding throughout the concentration range studied (5–200 mM), and SO_4^{2-} also caused a significant facilitation at 100 mM. It should be noted, however, that the rank orders of potency of the chaotropic properties are modified by several factors and are not invariant⁵. As an example of such a situation we may cite the work of Collins and Salton⁶ in which 100 mM $\text{Ba}(\text{SCN})_2$ is nearly as effective as 1% Triton X-100 in solubilising mannan from *Micrococcus lysodeikticus* membranes while being almost completely ineffective in the case of

Table 1 Correlation between the facilitation of ^3H -diazepam binding and ion concentration

Ion	Area under specifically bound ^3H -diazepam curve (0–200 mM arbitrary units)	Weighted half time to equilibrium ³ ($T_{\text{Cl}}/T_{\text{A}}$)	% Reversals ³
Br^-	704	1.04	100
I^-	650	2.24	93
NO_3^-	403	0.79	75
Cl^-	399	1.0	100
F^-	258	—	33
IO_3^-	173	—	0
SCN^-	165	1.98	67
ClO_3^-	126	0.41	80

Diazepam binding assays were carried out with well washed whole brain (less medulla-pons). Membrane fragments were prepared as described previously⁷. Membrane fragments equivalent to 125 μg protein were incubated on ice with 3 nM ^3H -diazepam in a total volume of 1 ml 100 mM Tris-citrate buffer, pH 7.1, and nonspecific binding was determined by displacement of ^3H -diazepam with 3 μM clonazepam. Sodium salts were used throughout with the exception of NH_4SCN , and compared with the maximal level of facilitation, inhibition was found with I^- at 200 mM and SCN^- at and above 50 mM. Areas under the curves were estimated by triangulation and even if SCN^- is omitted, because of its marked inhibitory activity, correlation coefficients do not increase markedly. Attempts to draw correlations between the electrophysiological data and the lowest anion concentrations required to produce a significant facilitation of ^3H -diazepam binding produced even weaker correlation coefficients.

cytochrome *c*. Until we are more familiar with the characteristics of this membrane preparation it would be unwise to draw any conclusions about the effects of chaotropic ions upon it.

We feel that the data so far available are insufficient to distinguish adequately between the possible explanations available for the facilitation of ^3H -diazepam binding caused by certain anions, and that it is premature to infer a direct linkage from benzodiazepine receptor to a chloride ion channel.

J. M. CANDY

I. L. MARTIN

*MRC Neuropharmacology Unit,
Department of Pharmacology,
The Medical School,
Birmingham, UK*

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RODBARD, COSTA AND PERT
REPLY—In our recent study¹, only five anions (I^- , Br^- , Cl^- , NO_3^- and SCN^-) of a series of 18 tested increased the affinity of diazepam binding to rat brain membranes.

Confirming the report of Tallman *et al.*², Martin and Candy³ have reported the enhancement of diazepam binding by γ -aminobutyric acid (GABA) and have further noted the facilitatory effect of NaCl and KCl on the GABA-enhanced binding. However, we believe that their attempt to correlate the binding data with the electrophysiological data of Kelly *et al.*⁴ is spurious for the following reasons. (1) The area under those biphasic binding curves has no apparent physicochemical meaning; (2) the anions have variable inhibitory effects; (3) Kelly *et al.*⁴ pointed out that the relationship between percentage reversal and anion mobility ‘is clearly not a simple one’, and that the $T_{\text{Cl}}/T_{\text{A}}$ equilibrium ratios ‘only give an approximate estimate of the relative mobilities’.

It seems reasonable to view the facilitatory ion effect of anions on binding as an all or none phenomenon, as Eccles⁵ described anion channel permeability. Viewed this way, it is striking that the same five anions which detectably enhance diazepam binding are the five most active anions according to Kelly *et al.*⁴. In fact, the probability of this being coincidental is about 1 in 1,000.

The possibility that these anions are effective by virtue of a nonspecific chaotropic effect is extremely unlikely in our view: (1) the active anions displayed an ED_{50} in the range of 2.8–20 mM, concentrations far below those usually necessary to elicit chaotropic effects; (2) strong chaotropic agents (guanidinium) are inactive or even decrease binding of diazepam to its receptor (unpublished data); (3) anti-chaotropic agents (fluoride, sulphate, phosphate) do not reverse the iodide enhancement of diazepam binding.

In conclusion, we agree with Martin and Candy's contention that differential linkage of neurotransmitter receptors with the same anion channel is a possibility. In fact, we have previously interpreted our finding⁶ of large regional differences in the ability of iodide and GABA to enhance diazepam receptor binding as indicative of differences in the extent to which GABA and diazepam receptors share the same ionophore.

Finally, we report new data which we are preparing for publication. The compound 4,4'-diisothiocyano-2,2'-stilbene disulphonic acid (DIDS) is a specific anion channel blocker in the erythrocyte membrane⁷, which has an anion specificity similar to that of cortical neurones. In competition experiments, DIDS can completely reverse the enhancement of diazepam binding by active anions at concentrations (ED_{50} = 100 μM) which have no effect on control binding.

Although definitive proof of the linkage of benzodiazepine receptors with anion channels will require further electrophysiological substantiation and structural biochemical evidence, we believe that the benzodiazepine binding data

strongly support the notion⁶ that benzo-diazepine, GABA and perhaps glycine receptors interact with the same chloride ion channels.

DAVID RODBARD
TOMMASO COSTA

*Endocrinology and Reproduction
Research Branch,
National Institute of Child Health
and Human Development*

CANDACE B. PERT

*Section on Biochemistry
and Pharmacology,
Biological Psychiatry Branch,
National Institute of Mental Health,
Bethesda, Maryland 20205*

- Costa, T., Rodbard, D. & Pert, C. B. *Nature* **277**, 315–317 (1979).
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Another interpretation of communal breeding in green woodhoopoes

LIGON AND LIGON¹ presented information which they stated "suggests helping is a strategy for personal gain", and concluded that their observations "do not support an interpretation of helping behaviour relying on kin-selected altruism." Obtaining critical evidence on these two points is difficult, perhaps justifying presentation of preliminary data. Nevertheless, it should be pointed out that their results are open to other interpretations and, in my view, they are entirely consistent with my proposed theory² incorporating kin selection and individual selection, which they rejected.

Ligon and Ligon apparently invoked the personal-gain theory because the helpers showed no evidence of discrimination in their feeding of nestlings according to genetic relatedness. They did not actually demonstrate any personal gain; they merely inferred that it must exist because they saw that some helpers were not related to the young they fed. I consider this to be insufficient evidence on which to base the interpretation of personal gain. The few data presented are equally compatible with a zero-gain or even a loss (that is, altruism) hypothesis, as long as, on average, helping causes a gain in inclusive fitness to the helper. The apparent eagerness of helpers to feed young could be similarly explained.

Although personal gain to helpers by helping has been invoked by many authors and seems highly likely (for review see ref. 3), I know of no case in which a personal gain in terms of direct (individual) fitness

has been convincingly demonstrated. Although there is some evidence for this⁴, firm empirical data based on a strong-inference hypothesis are lacking. Apparently, Ligon and Ligon considered that their observations of a few helpers feeding unrelated young was inconsistent with kin-selection theory. They did not give their reasoning, but it seems to be based on two points: lack of discrimination of genetic relatedness in feeding efforts, and presence of presumed personal gain.

Discrimination was not evident in the few cases described, but the sample is unfortunately quite small (three cases). It may be of interest that a similar, though insignificant, lack of discrimination has been shown in jays (Fig. 1 of ref. 5). Even some parents in communal birds sometimes fail to discriminate between their own young and that of others (p. 375 of ref. 6), but one would not interpret this as evidence that parents in general have no genetic interest in their young. In any case, discrimination is not a necessary feature of kin selection^{7–12}, even though some authors have written as if it were¹³.

Personal gain, although inconsistent with altruism, is entirely consistent with kin selection. This is not always evident in writings on kin selection^{13,14}, but, as clearly shown by Hamilton¹¹, the concept of inclusive fitness specifies that genetic costs and benefits be accounted across all relatives (not just offspring), regardless of the sign of the effect. My theory² for the evolution of helping was based on a combination of kin selection and individual selection. Although I called helpers 'altruists', altruism or sacrifice by the helper was not a necessary or even desirable part of the theory, which is equally applicable when personal gain to the helper is involved.

As kin selection requires neither discrimination by relatedness nor individual sacrifice, it is difficult to see how the data of Ligon and Ligon are inconsistent with kin selection. The important condition is that the indirect fitness (inclusive fitness minus direct or individual fitness¹²) be raised by helping, and the Ligon did not consider this point.

JERRAM L. BROWN

*Department of Biological Sciences,
State University of New York,
Albany, New York 12222*

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LIGON AND LIGON REPLY—Brown's major criticism of our report¹ is that we rejected his theory² incorporating both individual and kin selection in the evolution of avian helper systems. Actually, we questioned only kin-selected or phenotypic altruism^{3,4} in one kind of bird. Because green woodhoopoes can gain breeding status and helpers by first being helpers, whether or not they are related to the nestlings aided, we suggested that helping is a strategy for personal gain. For example, older birds gain and hold new breeding positions as a result of the support provided by younger individuals⁵. The system we describe corresponds to Hamilton's⁴ selected category and can also be referred to as cooperation⁶, reciprocal altruism⁷, reciprocity³ or mutualism⁸. Kinship ties may or may not be present but are not required for this kind of behavioural interaction.

Brown proposes alternative explanations for our observations. We feel that because of the many unknown factors related to kin-selection theory⁹ the most parsimonious explanation for avian helping behaviour is personal or direct gain¹⁰, when this can be ascertained. Very recently, Brown¹¹ has acknowledged that kin selection may not always be involved in communal helper systems, and Brown and Brown¹² have urged abandonment of the term 'altruism' to refer to avian helping behaviour. Both points are addressed in our report and we support both suggestions.

J. DAVID LIGON
SANDRA H. LIGON

*Department of Biology,
The University of New Mexico,
Albuquerque, New Mexico 87131*

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reviews

Scientific R & D in France

R.W. Cahn

Le Pouvoir et la Science en France. By Pierre Papon. Pp. 315. (Editions Centurion: Paris, 1979.) Paperback Fr.65.

THIS book is one of the few detailed studies of the politics of scientific research and development in France. The author is professor of physics in one of the Grandes Ecoles, has been involved in the central planning machinery of French government, and is active in advising the French Socialist Party on matters of scientific research.

Professor Papon is extremely informative — the book is full of historical facts and figures; he is critical of French administrative procedures and again backs his criticism with much historical evidence; and in the last third of the book he steps back from the here and now to analyse, with an impressive grasp, the problems of method and objective involved in attempting to plan scientific research at all. Starting with the ideas of Bernal (from whose *Social Functions of Science* he quotes a long passage) he analyses very fairly the temptations, pitfalls and possible benefits of planning applied to science and development, and faces the inescapable conflicts between scientific research perceived as an autonomous liberal profession and science as a servant of the Commissariat au Plan.

An initial chapter reviews the history of the scientific profession in France; there is a tantalising glimpse of 'le grand patron' (he says of Pasteur that "il ne put faire triompher ses idées qu'une fois qu'il eût vaincu le scepticisme d'un patron important"), but that crucial species is not further analysed. Professor Papon then goes on to recount in detail the development and repeated changes in central scientific administration. As in Britain, the changes were many. A wilderness of advisory committees, both general and specialised, interministerial committees and delegations waxed and waned (though some, particularly the Committee of the Twelve Wise Men of which Professor Papon was a member, have shown great staying power). The CNRS, which sets up and finances research teams, and the DGRST, which administers studentships, fellowships and grants, together fulfil the functions of the SRC in Britain. Last year, the various bodies and committees were brought together under the aegis of a specialised Minister of Science — a

condition which, for better or worse, Britain has not yet reached.

The author goes on to analyse in some depth the position of scientific research in universities and the Grandes Ecoles. Although recognising the major stimulus CNRS has given French science, he criticises it for having sapped the scientific independence of academic institutions: many CNRS laboratories are off-campus. The engineering Ecoles come in for special condemnation for having neglected research, and this is partly laid at the door of CNRS for being very fundamentally oriented, and partly attributed to the mathematical and analytical bent of the Ecoles, beginning with the nature of their competitive entrance examinations.

A substantial part of the book is devoted to research in industry and in the large quasi-industrial state institutions, especially the Commission à l'Energie Atomique, whose history is recounted in detail. This topic leads Professor Papon on to analyse de Gaulle's hostility to supranational (as opposed to bilateral) scientific arrangements, the cause of his unrelenting persecution of Euratom. A preference for bilateral or trilateral accords survives today: sometimes it works very well, as with the Airbus Consortium; sometimes badly, as with French attempts to foster the computer industry (again the story is told fully). Perhaps the crowning irony in French technological history, as Professor Papon describes, was the CEA's decision in 1969 to replace the home-grown graphite/gas reactors with American-style light-water reactors; Britain, which is more open to American suasions in general, has not yet been persuaded.

For a British reader, perhaps the most surprising claim made by Professor Papon is that it is virtually impossible for a research scientist or engineer to penetrate the top echelons of industrial management. He tells us that the R&D laboratory is a place of exile eschewed by ambitious would-be industrialists, and many Polytechniciens and other graduates of Grandes Ecoles, though trained formally as engineers, go straight into administration. We are often told in Britain that the French approach to engineering education as an elite enterprise ensures proper recognition of engineering skills at the highest level. Professor Papon's arguments suggest that this is only so if the engineers contrive to forget their professional origins. It would be instructive to have chapter and verse, and perhaps the Finiston Committee in the UK will provide it as part of their international comparisons.

Only part of one sentence in the book is devoted to the influence of research workers' trade unions, of which there are several in France. Could this have happened if the book had been written by an English socialist?

Professor Papon is often hard on his fellow-countrymen and on his country's institutions, but he generally documents his assertions, his reading has been catholic, and he freely makes international comparisons. This modestly priced book will be of interest and value to all those concerned with science politics, whatever their nationality. □

R. W. Cahn is Professor of Materials Science at the University of Sussex, Brighton, UK.

Marine communities

Biogeography and Adaptation: Patterns of Marine Life. By G. J. Vermeij. Pp. 332. (Harvard University Press: Cambridge, Massachusetts, and London, UK, 1978.) £17.50.

THIS thought-provoking book is mainly concerned with the influence of biological interactions, especially predation, on the architecture of hard-shelled marine

organisms and on the stability and biotic history of marine communities. It is divided into three main parts and is followed by an appendix, comprehensive reference list and index. The first part points out the occurrence of gradients in shell form of gastropods, bivalves and other organisms along latitudinal gradients and with depth below the high water mark. The author suggests that these primarily reflect anti-predatory defences which are developed to a greater degree and are found in an increasing proportion of species along a gradient from high to low

latitudes. The second part is concerned with inter-oceanic differences in the degree to which predation-related adaptations in shell structure are developed, and the third part reviews inter-oceanic differences in patterns of extinction and speciation along latitudinal gradients.

One has the impression that the author has drawn on a considerable personal experience of marine communities in many parts of the world and although most of the examples are taken from the Mollusca, particularly from the shelled gastropods, this does not seriously detract from the fundamentally interesting ideas which emerge from the text. The main problem with the book is that in many ways the intuitive ideas expressed in the text are ahead of the data necessary to support them. This is due in part to the difficulty of quantifying architectural differences in shell type and partly to the global comparisons ranging through geological time. Nevertheless the trends are illustrated as far as possible by profuse photographs, although the uninitiated (including myself) may find some of these difficult to interpret.

The chapter on vulnerability to extinct-

ion in stress-tolerant, opportunistic and "biologically competent" species and its relevance to biogeography throughout an evolutionary time scale will be of particular value to scientists and senior students with interests in palaeo-biogeography. Nevertheless, I personally would have felt the work as a whole to have been more satisfying if there had been some more serious attempt to quantify the trends which are illustrated in the photographs. As the author himself admits frequently in the text, a great deal more data need to be obtained to verify some of the ideas raised in the book. However, informed speculation, particularly when expressed in a thoroughly readable and lucid style as it is in this book, can play an important part in promoting new work on the nature of animal adaptation. In this respect the book is a valuable addition to the literature and represents a significant contribution to our understanding of the ways in which biological interactions influence the structural design of living organisms.

R. C. Newell

R. C. Newell is Visiting Professor at the University of Cape Town, South Africa.

Radiation damage in semiconductor materials

Radiation Effects in Semiconductors and Semiconductor Apparatuses. By V.S. Vavilov and N.A. Ukhin. Pp. 280. (Consultants Bureau/Plenum: New York and London, 1978.) £30.

AN English translation of a scientific book by an authoritative Russian author is always of interest as an insight into Russian thinking. When the subject has strong connections with technological fields such as semiconductors and nuclear science, the interest is even greater. It is thus a disappointment to find that the original of this book was finished over ten years ago — much too long ago in a fast-developing subject such as this. Viktor Vavilov has done distinguished work in the field of radiation damage in silicon, especially in the fields of photoconductivity and photo-voltaic effects, and such work requires an extensive background knowledge of radiation damage in bulk silicon and germanium. The surveys of these latter topics are useful, especially as the classic work in this field was done before 1967. Moreover, the accounts given of the carrier removal rates and other defect phenomena in these semiconductors include summary tables which can be used by research workers for a long time. On the other hand, the fact that the semiconductor technology discussed is over 12 years old lends to other parts of the book an

overwhelming impression of ancient history. In addition, the crediting of work to various authors is vaguely done, so that many sections do not even serve as historical guide. No specifically Russian insights are revealed, as much of the best work discussed is American. Evidence that the present anonymous translator was not an expert in the field comes from the mangled versions of US workers' names (Prines for Pines; Byub for Bube, and so on — products of translation into Russian and then back to English). "Apparatus" in the title should, of course, read "Device".

The surveys of radiation damage and transient radiation effects seem fairly complete, except for a curious (even political?) omission of the work of Rappaport, Wysocki and co-workers on lithium doping in silicon. The discovery by this group of the action of lithium in the 'healing' of radiation damage in solar cells was an astonishing technological application of basic radiation damage physics. Could it be that Vavilov does not wish to admit that, although engrossed in basic studies of lithium in damaged silicon, his group missed this particular trick; or is there some defence application of the principle which puts the work outside the realm of discussion in the USSR?

Due to lack of other good summaries of the field, this book will find a place on the shelves of research workers, but its main rôle is that of an historical record.

Andrew Holmes-Siedle

Andrew Holmes-Siedle is Consultant to the Physics Department of the Fulmer Research Institute (Slough, UK) in the fields of solid-state physics and radiation physics.

Mechanical properties of ceramics

Mechanical Behaviour of Ceramics. By R.W. Davidge. Pp. 165. (Cambridge University Press: Cambridge, 1979.) £15.

THIS book fulfils a need that has existed for some time now. The mechanical properties of ceramics have been a subject of comparatively intense research for some two decades or more — in fact, ever since the 1950s, when the hope was expressed that these brittle materials might be made tougher by more research, the optimism centring initially at any rate on the prospects for improvements in the ductility of polycrystalline ceramics by analogy, I suspect, with the history of improvements to cast iron. As becomes apparent from reading this book these hopes were not well founded. It would be hard to find a better author for this book than Dr Davidge who has been at the forefront of research in this subject for numerous years.

The layout of the book is essentially classical, mapping out our knowledge of the factors affecting the intrinsic elastic and strength properties of ceramics, the treatment of flaws through fracture mechanics and finally reviewing the essential details of plasticity and the reasons for many ceramics being inherently strong. These areas are reviewed in the first 5 chapters of the book.

Chapters 6-9 deal with the fracture strength, impact resistance, toughness, thermal shock, and last but not least, engineering design data. The scientific arguments are well presented and the accent is inevitably on the so-called engineering ceramics which are comparatively simple ones, usually single-phase polycrystalline and low in porosity. I suspect that the industrial ceramist who is often dealing with multi-phase, inhomogeneously textured ceramics often containing appreciable porosity, may view the book critically. For example, he will not find any comment on the well established strength/porosity relationships. Neither is there any attempt to demonstrate how the knowledge developed can be used to design a ceramic. No doubt Dr Davidge takes the view that our understanding has not developed sufficiently to take such issues on board.

However, the above remarks are not meant to detract from the excellence and usefulness of this book which I am sure will be welcomed by the scientist researching into the subject.

J.E. Bailey

J.E. Bailey is Professor of Materials Technology at the University of Surrey, Guildford, UK.

The first nanosecond

Chlorophyll Organization and Energy Transfer in Photosynthesis. Ciba Foundation Symposium (new series). Edited by G. Wolstenholme and D. W. Fitzsimons. (Excerpta Medica: Amsterdam, Oxford and New York, 1979.) \$38.25; Dfl86.

THIS book presents the proceedings of a meeting held in February 1978, on the primary processes of photosynthesis. The events in question span the time from the moment when light is absorbed, to the first separation of electric charges. All this happens in less than a nanosecond (10^{-9} s). The subsequent processes of biochemical electron transfer were deliberately excluded from consideration in this book. Those readers who find this too restrictive might consult *Primary Processes of Photosynthesis (Topics in Photosynthesis, Vol. 2, edited by J. Barber; Elsevier: Amsterdam, New York and Oxford, 1977)* which takes a broader view of the primary processes, at least as far as the millisecond time scale.

The present book is principally concerned with that remarkable molecule, chlorophyll and the way in which it is

arranged in the chloroplast membranes to give the properties that are required for photosynthesis. In fact chlorophyll seems to exist in several types of proteolipid complex, each having a different function. The bulk of the chlorophyll serves as antenna to capture light energy and rapidly transfer it to reaction centres. The spectroscopic work of Katz and his group has convincingly shown that each reaction centre contains a special pair of chlorophyll molecules. The next step is presumed, by analogy with the simpler systems of photosynthetic bacteria, to be the formation of a radical pair in the reaction centre. From then onwards, the rest is chemistry. The details of these processes are being investigated from various directions. The structure of the system can be investigated by electron microscopy, and by biochemical investigation of the chlorophyll-protein complexes. The dynamics of energy transfer is investigated by spectroscopic techniques; at present only optical methods are fast enough. The most spectacular is laser-pulse spectroscopy which makes it possible to study fluorescence lifetimes of the order of 10^{-11} - 10^{-10} s. 'Picosecond' has become something of a buzz-word in this field. By this means the initial processes of energy transfer are now accessible, if the results are compared with suitable

chemical and/or theoretical models.

The papers are interspersed with long discussions, (which even have their own reference lists and index). These are one of the most valuable features of the book, for here the experts on these disparate methods are trying to reconcile their results to give a coherent picture. How can the chlorophyll-protein complexes extracted by Thornber, Anderson and others, be identified with the various lumps seen in the chloroplast membranes in the freeze-etch electron micrographs of Staehelin and Arntzen? How does the theory of exciton transfer allow energy to be transferred from them to the reaction centres? Perhaps there is more chlorophyll in between, and so on.

The view of the photosynthetic unit which this book takes as its starting-point is considerably more advanced than it was a few years ago. It is possible, as Sir George Porter suggests in his Chairman's introduction, that a fairly complete understanding may emerge in the next few years. We are not there yet, but the book gives an indication of the techniques by which this understanding may be achieved.

Richard Cammack

Richard Cammack is Lecturer in the Department of Plant Sciences, King's College, University of London, UK.

Electrical phenomena in polymeric materials

Electrical Properties of Polymers. By A. R. Blythe. Pp.191. (Cambridge University Press: Cambridge, 1979.) £17.50.

THIS book provides an excellent basic introduction to a wide range of electrical phenomena in polymeric materials in terms of the molecular and electronic processes involved. It brings together the well-established area of dielectric relaxation, which has contributed greatly to our understanding of molecular conformations and correlated motions in polymers, with the less well understood phenomena of conduction and electrostatic charging, each of which are assuming increasing importance because of their relevance to polymer applications.

The introductory chapter includes a brief survey of the chemical and physical structure of polymers, providing a useful background to those with limited experience of polymer science. In chapter 2 the derivations of standard relationships between the equilibrium dielectric constant and molecular dipole moment are clearly

outlined. The third chapter introduces and defines the frequency-dependent dielectric constants and discusses the associated relaxation parameters and underlying molecular reorientational processes. Chapter 4 presents a comprehensive account of dielectric measurement techniques. An illuminating discussion of current knowledge and ideas regarding ionic and electronic conduction mechanisms is given in chapter 5 together with relevant sections on conducting composites and resistivity measurements and some interesting suggestions on the feasibility of obtaining materials with novel conduction properties. The two final chapters are concerned with the practically important topics of dielectric breakdown and static charge phenomena, respectively.

The book is written with care and authority and is particularly recommended to students of polymer science as an educative text on electrical behaviour. Polymer research workers, industrial manufacturers and users of plastics should also find the book a useful acquisition, bearing in mind the detailed accounts of measurement techniques, the references to further reading at the end of each chapter and relevance of electrical properties to polymer applications.

B.E. Read

Bryan E. Read is in the Division of Materials Applications of the National Physical Laboratory, Teddington, UK.

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Raman effect

The Scientific Papers of C. V. Raman. Vol. 1: The Scattering of Light. Pp. 699. (Indian Academy of Sciences: P.O. Box 621, Bangalore 560 006, 1978.) £8

THE Raman effect is one of the most significant discoveries ever made in spectroscopy. Professor Sir C. V. Raman (1880–1970) was awarded the Nobel prize for physics in 1930 for his work on the scattering of light which resulted in this discovery.



Sir C. V. Raman

This clearly produced volume contains a selection of 94 of the scientific papers published by Professor Raman, either by himself or along with his students, on the subject of light scattering. It contains an historical introduction to the life and work of this "grand old man" of Indian Science by S. Ramaseshan who was responsible for selecting and editing the papers on behalf of the Indian Academy of Sciences. The collection contains papers on colloid scattering, molecular scattering, surface scattering and Brillouin scattering and also his pioneering publications on molecular anisotropy.

The monograph he wrote in 1922 on the *Molecular Diffraction of Light* and also his investigations on X-ray scattering and Compton scattering have been included, as these played a significant role in his discovery of the Raman effect in 1928. The intense interest he developed in the scattering of light was due to the visual impact the blue of the Mediterranean sea made on him on his first voyage to Europe in 1921. Even on this journey he was well prepared for experimental observations, for he carried in his pocket Nicol prisms, a small telescope to which polarisers and analysers could be attached, a slit, and even a diffraction grating. In later years he studied the scattering from pure organic materials which S. Venkateswaran, a part-time worker in his laboratory, had succeeded in purifying by slow distillation *in vacuo*. Using filtered sunlight to irradiate these liquids in his work with K. S.

Krishnan, Raman observed that all pure organic liquid showed this "feeble fluorescence" and he became convinced that this was the modified scattering of altered wavelength corresponding to the "milder fluctuations" in the state of the scattering molecule. Pointing a direct vision spectroscope on to the scattered track he saw that the scattered light contained not only the incident colour but at least another separated in wavelength from it by a dark space. Then replacing the filtered sunlight by a mercury arc he recorded sharp modified Raman lines for which the frequency shifts were identified with some of the characteristic infrared active vibrational frequencies of the molecule.

He was a man of wide interests in physics and published many papers on sound, ultrasonics, the solid and liquid state as well as optical phenomena. His physical intuitive abilities enabled him to jump several steps in mathematics and his capacity to expound complicated concepts in physics in a simple and appealing manner always made him a popular speaker. The lecture in which he announced his discovery of the Raman

Effect and his Nobel lecture are included in the volume.

Sir C. V. Raman lived long enough to see the whole field of Raman spectroscopy rejuvenated and increased in importance by the use of lasers, which form ideal sources for the observation of the effect. This has enabled much of the earlier work to be repeated under conditions of greater resolution and signal to noise ratio. However, this volume reminds us of the way that Raman blazed new trails by discovering new phenomena such as shear waves in liquids and the 'soft mode' process in crystals. This book will be of value to all interested in light scattering and the Raman effect. At £8 it represents outstanding value for money as it is a bound volume of nearly 700 pages. Along with the International Raman conference held last year in Bangalore this record of his work forms a fine tribute to the achievements of this outstanding Indian scientist.

G. R. Wilkinson

G. R. Wilkinson is Professor of Physics at King's College, University of London, UK.

Marine productivity

Marine Production Mechanisms. International Biological Programme, Vol. 20. (Edited by M. J. Dunbar. Pp. 338. (Cambridge University Press: Cambridge, 1979.) £25.

LONG before the International Biological Programme (IBP) was thought of, international collaboration was a feature of marine science and it is probably because of this that this synthesis volume from the marine productivity section does not survey the whole field but presents a number of largely unrelated chapters summarising work carried out specifically under IBP auspices. The contributions fall into two groups, regional studies of productivity and ecosystem structure, with an *entr'acte* in the form of a short chapter by Velasquez on seaweed utilisation in the Philippines.

The regional studies span seas from the sub-Arctic — primary production in Frobisher Bay, Arctic Canada, is described by Grainger — to the tropics — primary productivity in Indian waters being reviewed by Qasim. Detailed studies of food webs in the coastal waters of Japan are described by Hogetsu, the vertical distribution of phytoplankton in the open Pacific and Atlantic oceans by Semina, and production cycles in the Dutch Wadden Sea by van der Eijk. Parsons touches on mathematical modelling in his account of the Strait of Georgia, British Columbia, but there is no extensive discussion of the potentialities of this approach. Grindley discusses productivity and upwelling in the Benguela region and Dunbar relates

production in the Gulf of St Lawrence to the pattern of water circulation, but the currently fashionable topic of the relationship of production to fronts in the sea has emerged since the IBP was concluded.

The contributions on ecosystem structure are of particular interest in being all by Russian workers. Pepita gives a quantitative account of energy flow in food webs in southern seas of the USSR and the tropical Pacific. A major role in energy supply is attributed to bacterial utilization of dissolved organic matter in tropical waters both by Pepita and by Shushkina and Vinogradov in the following chapter; the contributors to the earlier chapters largely assume that the classical radiocarbon technique of Steemann Nielsen gives an adequate measure of primary production. Neyman and Yablonskaya in the two next chapters employ the useful concept of trophic groups in studies of benthic faunas, and finally Golikov and Scarlato describe the pattern of distribution of benthic faunas in the shelf seas of the USSR. All this is most interesting but few details of methods are given so that, the references being almost exclusively to papers in Russian journals, assessment of the results will be difficult for most of us.

This book will be of great value to the specialist but cannot be recommended as an introduction to the study of marine productivity.

G. E. Fogg

G. E. Fogg is Head of the Department of Marine Biology at the University College of North Wales, Bangor.